FORMULATION, EVALUATION AND COLON TARGETING OF 5-FLUOROURACIL NANOPARTICLES USING pH SENSITIVE POLYMER

Dissertation work submitted to
THE TAMIL NADU Dr. M.G.R. MEDICAL UNIVERSITY, CHENNAI

In partial fulfillment of the award of degree of

MASTER OF PHARMACY

(Pharmaceutics)

Submitted by
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March 2009

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Certificate

This is to certify that the dissertation entitled **FORMULATION, EVALUATION AND COLON TARGETING OF 5-FLUOROURACIL NANOPARTICLES USING pH SENSITIVE POLYMER,** was carried out by **JAYESH.V.N.**, in the Department of Pharmaceutics, PSG College of Pharmacy, PSG Institute of Medical Sciences & Research, Peelamedu, Coimbatore, which is affiliated to The Tamilnadu Dr. M.G.R. Medical University, Chennai, under the direct supervision and guidance of Dr. C. Vijaya Raghavan M.Pharm., Ph.D., Vice Principal & Head, Department of Pharmaceutics, PSG College of Pharmacy, PSGIMS & R, Coimbatore.

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ACKNOWLEDGEMENT

I take this opportunity with pride and immense pleasure in expressing my deep sense of gratitude to **Dr. C. Vijaya Raghavan M.Pharm., Ph.D.**, Vice Principal and Head, Department of Pharmaceutics, PSG College of Pharmacy, PSG IMS&R, Coimbatore, whose guidance was unforgettable, invaluable. His impressive, innovative ideas and constructive suggestion has made the presentation of my work a grand success.

My sincere gratitude to our beloved Principal **Prof..A.K.Chandrasekharan M.Pharm.,** PSG College of Pharmacy, PSG IMS&R, Coimbatore, for providing every need from time to time to complete this work successfully.

I submit my sincere thanks to our beloved Managing Trustee of PSG Sons and Charities, **Shri. G.Rangaswamy** for providing all the facilities to carryout this work.

I express my heartfelt sincere thanks to **Mr. S. M. Habibur Rahman M.Pharm (Ph.D),** Lecturer Department of Pharmaceutics, PSG College of Pharmacy, PSG IMS&R, Coimbatore, for his valuable suggestions and support during this study.

I am elated to place on record my profound sense of gratitude to **Mr. V.Sankar, M.Pharm., (Ph.D)., Mrs.S Durgaramani, M.Pharm. (Ph.D.,),Assistant Professors** and **Mr.S.Subhramanyam**,**M.Pharm., (Ph.D.,),** Lecturer, Department of Pharmaceutics, PSG College of Pharmacy, PSG IMS&R, Coimbatore, for their constructive ideas at each and every stage of the project.

I owe my gratitude and special thanks to **Ms. K.Y.Kavitha,M.Pharm.,** Assistant Professor**,** Department of Pharmaceutical Analysis, PSG College of Pharmacy, PSG IMS&R, Coimbatore, for helping me to carryout the analytical and interpretation studies.

I would like to thank **Mrs.R.Chithra, B.Sc. Bio Chemistry, Sr. Lab Technician,Ms. Sheela Gracy, D.Pharm., Lab Technician, Librarians** and **Non Teaching Staff** for their kind co-operation during this work.

It is privilege to extend my special thanks to **my dearest Lovable Parents,** without whose unconditional love and support this process of my learning would have been incomplete. And they are also the backbone for all successful endeavors in my life.

Words can't express my sincere gratitude and obligation to my dear batch mates **Harikrishnan.V, John.R, Prasath.R, Arunprasath.B, Siddharth, V.Harikrishnan, Muthukumar ,Velayutham K, Praveen .C** and to all other batch mates who directly helped during my work.

I would like to thank my roommate **Gladwin George and Prithviraj** who helped me to complete my project work.

I wish to extend my special thanks to my Junior friends **Jibin Joshy** and **Antony Nobi** for their kind help during my project work.

I would like to thank my **Juniors** and to all other batch mates who directly or indirectly helped during my work.

I wish to thank of **M/s. Saraswathi Computer Centre** for framing project work in a beautiful manner.

My sincere thanks to all those who have directly or indirectly helped me to complete this project work.

Above all, I humbly submit my dissertation work, into the hands of **Almighty**, who is the source of all wisdom and knowledge for the successful completion of my thesis.

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SCOPE AND PLAN OF WORK

Colorectal cancer is a very common malignancy in industrialized nations and a major cause of mortality and morbidity. Surgery, radiation therapy and chemotherapy are the three varieties of treatments commonly using for colorectal cancer. 5-Fluorouracil is one of the best drug of choice for colorectal cancer .It is also used for other types of malignancies such as breast cancer, head and neck cancer, because of its incomplete and erratic oral bioavailability. 5-Fluorouracil commonly administered intravenously, however, patients are showing more compliance on oral dosage forms rather than intravenous therapy, with oral treatment potentially more easy administration and less costly. The regiments include an intravenous bolus or continuous infusion of 5-Fluorouracil modulated with folinic acid (Leucovorin). On intravenous administration, 5-Fluorouracil produces sever toxic effects of gastrointestinal, hematological, neural, cardiac and dermatological origin. Site specific delivery of 5-Fluorouracil may reduce the systemic side effects and provide effective and safe therapy of colorectal cancer that may reduce the dose and duration of therapy when compared with the conventional treatment.

The approaches to achieving colonic delivery of drugs include use of prodrugs, pH sensitive polymer coatings, time–dependent formulations, bacterial degradable coating, and time/pH- controlled colon delivery capsules. A colon specific guar gum based tablet of 5-Fluorouracil has also been reported. However because of variations in transit throughout

the colon, the drug release can be impaired when the colon-specific tablet matrix is not readily disintegrated and treatment will remain ineffective. This problems could be circumvented by reducing, the size of the delivery carrier, since it has been reported that gastrointestinal retention depends upon the size of the carrier, meaning that smaller carrier will lead to longer residence in the colon.

In recent years, the interest in sub- micron systems (i.e.nanosystems) in pharmacy has urged. This is, in part, due to the potential advantage, these systems provide more conventional micro particulate systems. The challenges faced in the delivery of small and large molecules such as poor solubility, stability, and limited absorption can be over come by using nanosystems. Several anticancer drugs including paclitaxel and doxorubicin have been successfully formulated using nanotechnology.

The pH- dependent systems exploit the generally accepted view that pH of the human gastrointestinal tract increases progressively from the stomach (pH1-3), small intestine (pH6.5-7) to the colon (pH7-8). Most commonly used pH-dependent coating polymers are methacrylic acid copolymers-. Eudragit RL 100 and Eudragit S 100 which dissolve at pH 5.5, pH 6.0 and pH 7.0 respectively. The use of Eudragit RL100 prevented drug release in the upper GI tract, during intestinal passage and permitted selective drug delivery in the colon.

The present investigation involves formulation, characterization, vitro release studies and colon targeting of 5-Fluorouracil nanoparticles using Eudragit RL 100 for the treatment of colorectal cancer.

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INTRODUCTION

Nanotechnology is the science of matter and material that deals with the particle size in nanometers. Nanoparticles are solid colloidal particles ranging in size from 1 to 1000 nm (1 μm) and composed of synthetic or semi synthetic polymers (Vyas, 2002). Nanoparticles were developed around 1970s. They were initially devised as carriers for vaccines and anticancer drugs (Lijima,1991). In order to enhance tumor uptake, the strategy of drug targeting was employed, and as a first important step, research focused on the development of methods to reduce the uptake of the nanoparticles by the cells of the reticuloendothelial system (Edward, 2004). Nanoparticles were able to achieve, with success, tissue–targeting of many drugs. Nanoparticles are made up of non-biodegradable and biodegradable polymers.

The concept of drug targeting and controlled drug delivery can be achieved by surface modifications and choice of appropriate particle materials. Particles with magnetic characteristics can be retained at the target site by applying an external electromagnetic field. Nanoparticles composed of biodegradable polymers are proposed for application in oral chemotherapy. Nano sized micelles are of advantage for drug transports with the narrow therapeutic indexes. Because of their small size, nanoparticles are suitable for intravenous administration.

Nanotechnology is being employed in the pharmaceutical field for many reasons, but perhaps the leading goals are to improve drug solubility/bioavailability and/or delivery to various sites of action. Nanotechnology is also being employed to develop new and improved therapeutic devices. Nanotechnologies have already attracted over $3 billion of global government funding as part of efforts to enhance a range of disciplines including pharmaceuticals, drug delivery and healthcare monitoring. Advances in nanomaterials, nanostructures and nanosystems are expected to drive the value of the global nanotehnology market to over a trillion dollars by 2015. FDA is asking for pharmaceutical industry input to help the Nanotechnology Task Force refine its guidance recommendations. FDA wants advice on what characteristics of nanoparticles to consider when proving the safety and effectiveness of products containing nanoscale materials, and circumstances that would change a product's regulatory status due to the use of nanotechnology.

APPLICATIONS

Applications of nanotechnology in the different field can be summarized as follows:

Nanomedicines: Nanodrugs, medical devices, tissue engineering, etc.Chemicals and cosmetics: Nanoscale chemicals and compounds, paints, coating, etc.

Materials: Nanoparticles, carbon nanotubes, biopolymers, paints, coating.

Food science: Processing, nutraceutical food, nanocapsules

Envirnoment and energy: Water and air purification filters, fuel cells, photovoltics.

Military and security: Biosensers, weapons, sensory enhancement.

Electronics: Semiconductor chips, memory storage, photonics, optoelectronics.

Scientific tools: Atomic force, microscopes and scanning tunneling microscope.

Agriculture: Pesticides, food production

CLASSIFICATION OF NANOPARTICLES

In one dimensions (Thin surface coatings)

One-dimensional systems, such as thin films or manufactured surfaces.

1. **In two dimensions**

a) Carbon nanotubes

Carbon nanotubes are a new form of carbon molecule. Bound in a hexagonal network of carbon atoms, these hollow cylinders can have diameters as small as 0.7 nm and reach several millimeters in length . Each end can be opened or closed by a fullerene half-molecule. These nanotubes can have a single layer (like a straw) or

several layers (like a poster rolled in a tube) of coaxial cylinders of increasing diameters in a common axis.

2. In three dimensions

a) Fullerenes (Carbon 60)

Fullerenes are spherical cages containing from 28 to more than 100 carbon atoms. Fullerenes are a class of materials displaying unique physical properties. They can be subjected to extreme pressures and regain their original shape when the pressure is released. These molecules do not combine with each other, thus giving them major potential for application as lubricants.

b) Dendrimers

Dendrimers represent a new class of controlled-structure polymers with nanometric dimensions. They are considered to be basic elements for large-scale synthesis of organic and inorganic nanostructures with dimensions of 1 to 100 nm, displaying unique properties. Compatible with organic structures such as DNA, they can also be fabricated to interact with metallic nanocrystals and nanotubes or to possess an encapsulation capacity (Tomalia, 2004)

c) Quantum dots

It represents a special form of spherical nanocrystals from 1 to 10 nm in diameter. They have been developed in the form of semiconductors, insulators, metals, magnetic materials or metallic oxides.

3. Advantages of nanoparticles

- Increased bioavailability
- Dose proportionality
- Decreased toxicity
- Smaller dosage form (i.e., smaller tablet)

- Stable dosage forms of drugs which are either unstable or have unacceptably low bioavailability in non-nanoparticulate dosage forms.
- Increased active agent surface area results in a faster dissolution of the active agent in an aqueous environment, such as the human body. Faster dissolution generally equates with greater bioavailability, smaller drug doses, less toxicity.
- Reduction in fed/fasted variability (Edward, 2004)

NANOPARTICLE PRODUCTION PROCESSES

Nanoparticles can be produced by either dispersion-based processes (which involves breaking larger micrometer-sized particles into nanoparticles) or precipitation-based processes.

Dispersion-based processes

a) Wet milling

Wet milling is an attrition-based process in which the drug is dispersed first in an aqueous-based surfactant solution. The resulting suspension is subjected to wet milling using a pearl mill in the presence of milling media.

b) High-pressure homogenization

High-pressure homogenization is based on the principle of cavitation (i.e., the formation, growth, and implosive collapse of vapor bubbles in a liquid. In this process, a drug presuspension (containing drug in the micrometer range) is prepared by subjecting the drug to air jet milling in the presence of an aqueous surfactant solution.

The main advantage of high-pressure homogenization is that it is suitable for both large and laboratory-scale production because high-pressure homogenizers are

available in various sizes. In addition, homogenization creates negligible nanoparticle contamination, which is one of the most important objectives of a nanoparticle production process.

A limitation of this process is that the pressure used is so high that in some cases, the crystal structure changed.

c) Emulsification technology

Emulsification also can be used to prepare nanoparticle suspensions. In this method, the drug solution in an organic solvent is dispersed in the aqueous phase containing surfactant. This step is followed by the evaporation of organic solvent under reduced pressure, which results in the precipitation of drug particles to form a nanoparticle suspension which is stabilized by the added surfactant.

PRECIPITATION-BASED PROCESSES

a) Spray freezing into liquid (SFL)

In this process, developed at the University of Texas at Austin (Austin, TX) and commercialized by Dow Chemical Company (Midland, MI), an aqueous, organic, or aqueous–organic co-solvent solution; aqueous–organic emulsion; or drug suspension is atomized into a cryogenic liquid such as liquid nitrogen to produce frozen nanoparticles which are subsequently lyophilized to obtain free flowing powder.

b) Evaporative precipitation into aqueous solution (EPAS)

The EPAS process also was developed by the University of Texas at Austin and commercialized by Dow Chemical Company. In this process, the drug solution in a low boiling liquid organic solvent is heated under pressure to a temperature above the solvent's normal boiling point and then atomized into a heated aqueous solution containing stabilizing surfactant.

c) Rapid expansion from a liquefied-gas solution (RESS)

In an RESS process, a solution or dispersion of phospholipids or other suitable surfactant in the supercritical fluid is formed. Then, rapid nucleation of drug is induced in the supercritical fluid containing surfactant. This process allows rapid, intimate contact of the drug dissolved in supercritical fluid and the surfactant which inhibits the growth of the newly formed particles.

d) Precipitation with a Compressed Fluid Antisolvent (PCA)

In the PCA process (patented by RTP Pharmaceuticals and licensed to SkyePharma Plc., London, UK), supercritical carbon dioxide is mixed with organic solvents containing drug compounds. The solvent expands into supercritical carbon dioxide, thus increasing the concentration of the solute in the solution, making it supersaturated, and causing the solute to precipitate or crystallize out of solution.

Parameter	Characterization method(s)
Particle size and size distribution	Photon electron spectroscopy Laser defractometry Transmission electron microscopy Scanning electron microscopy Atomic force microscopy
Charge determination	Laser doppler anemomometry Zeta potentiometer
Surface hydrophobicity	Water contact angle measurements Rose Bengal (dye) binding Hydrophobic interaction chromatography X-ray photoelectron spectrometry
Chemical analysis of surface	Static secondary ion mass spectrometry, sorptometer
Carrier-drug interaction	Differential scanning calorimetry

Nanoparticle dispersion stability	Critical flocculation temperature
Release profile	*In vitro* release characteristics under physiological and sink condition
Drug stability	Bioassay of drug extracted from nanoparticles Chemical analysis of drug

HEALTH IMPLICATIONS OF NANOPARTICLES

It is important to differentiate between 'free' and 'fixed' nanoparticles. The former pose a more direct health threat because they are more difficult to contain, easily become airborne and can be inhaled.

Nanoparticles can enter the human body in several ways; (i) via the lungs where a rapid translocation through the blood stream to vital organs is possible, including crossing the BBB, and absorption by (ii) the intestinal tract, or (iii) the skin.

a) Skin

Particles 500–1000 nm in size, theoretically beyond the realms of nanotechnology, can penetrate and reach the lower levels of human skin, 128 and smaller particles are likely to move deeper into the skin. TiO_2 particles are often used in sunscreens to absorb UV light and therefore to protect skin against sunburn or genetic damage. Micrometer-sized particles of TiO_2 get through the human stratum corneum and even into some hair follicles – including their deeper parts. (Lademann et al., 1999).

b) Intestinal tract

The epithelium of the small and large intestines is in close contact with ingested material so that nutrients can be utilized.
A mixture of disaccharides, peptides, fatty acids, and monoglycerides generated by digestion in small intestine are further transformed and taken in the villi.

The kinetics of particle translocation in the intestine depends on diffusion and accessibility through mucus, initial contact with enterocyte or M-cell, cellular trafficking, and post-translocation events.

Charged particles, such as carboxylated polystyrene nanoparticles or those composed of positively charged polymers exhibit poor oral bioavailability through electrostatic repulsion and mucus entrapment. The smaller the particle diameter the faster they could permutate the mucus to reach the colonic enterocytes; 14 nm diameter permeated within 2 minutes, 415 nm particles took 30 minutes, while 1000 nm particles were unable to translocate this barrier

c) Lung

Based on three particle-types titanium dioxide (TiO_2), carbon black, hazard studies in rats demonstrated that ultrafine or nanoparticles administered to the lung produce more potent adverse effects in the form of inflammation and subsequent tumors compared with larger sized particles of identical chemical composition at equivalent mass concentrations or intratracheally-instilled doses. Surface properties, such as surface chemistry and area, may play a significant role in nanoparticle particle toxicity.

CLINICAL ASPECTS

Several nanoparticle technologies are currently in clinical trials and a few have progressed to clinical use. NanoCrystal™ technology from Elan Pharmaceuticals International Ltd. is one breakthrough technology that is being licensed to pharmaceutical companies for specialized drug delivery systems. Currently, there are some FDA approved drug products employing this technology. Rapamune (Wyeth-Ayerst Laboratories), an oral tablet dosage form containing nanoparticles of the immunosuppressant drug Rapamycin, was approved by the U.S. FDA

COLON

The large intestine is about 1.5 meters long, beginning at the caecum in the right iliac fossa and terminating at the rectum and anal canal deep in the pelvis. Its lumen is larger than that of the small intestine. It forms an arch round the coiled–up small intestine. For descriptive purposes the colon is divided into the caecum, ascending colon, transverse colon, descending colon, sigmoid or pelvic colon, rectum and anal canal.

The caecum is the first part of the colon. It is a dilated portion which has a blind end inferiorly and is continuous with the ascending colon superiorly. The vermiform appendix is a fine tube closed at one end, which leads from the caecum. It is usually about 13 cm long and has the same structure as the walls of the colon but contains more lymphoid tissue.

The ascending colon passes upwards from the caecum to the level of the liver where it bends acutely to the left at the hepatic flexure (right colic flexure) to become the transverse colon.

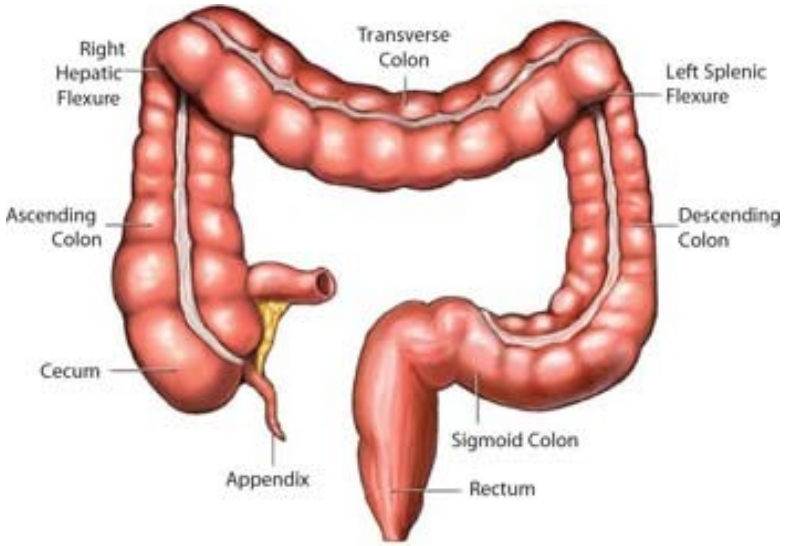
The transverse colon is a loop of colon which extends across the abdominal cavity in front of the duodenum and the stomach to the area of the spleen where it forms the splenic flexure (left colic flexure) by bending acutely downwards to become the ascending colon.

The descending colon passes down the left side of the abdominal cavity curves towards the midline. After it enters the true pelvis it is known as the sigmoid or pelvic colon.

The rectum is a slightly dilated part of the colon which is about 13 cm long. It leads from the sigmoid colon and terminates in the anal canal.

The anal canal is a short canal about 3.8 cm long in the adult and leads from the rectum to the exterior. There are two sphincter muscles which control the anus; the internal sphincter, consisting of stomach muscle fibers, is under the control of the autonomic nervous system and the external sphincter, formed by striated muscle, is under voluntary nerve control.

Fig. 1 Anatomy of large Intestine



Anatomy of Large Intestine

ANATOMY OF COLON

The colon is mainly situated in the abdomen. It is a cylindrical tube which is lined by a moist, soft pink lining called the mucosa; the pathway is called the lumen and is approximately 2-3 inches in diameter. The colon form the lower part of the gastrointestinal tract and extends from the ileocaecal junction to the anus .The colon is upper five feet of the large intestine and the rectum is the lower six inches. The junction of the small intestine (ileum) and the colon is in the lower right abdomen. The next portion of the colon, in the order in which contents flow, is the angle or bend is known as the hepatic flexure, located just beneath the rib cage. The colon then turns to a long horizontal segment, the transverse colon, beneath the left rib cage, the colon turns downward at the splenic flexure, to become the descending (distal) colon. In the left lower portion of the abdomen, the colon makes an S-shaped curve from the hip over the midline known as sigmoid colon. Lymph nodes are structures found in the circulating lymphatic system of the body that produce and store cells that fight infection, inflammation, foreign proteins and cancer.

PHYSIOLOGY

Approximately 500 ml of food pass through the colon daily. The various sections of the digestive tract absorb and remove water, propel waste throughout the long system of muscular tubes, work to keep the body alkalized, and accommodate the colonization of billions of beneficial microorganisms to aid us in breaking down waste matter.

BLOOD SUPPLY

Arterial supply is mainly by the superior and inferior mesenteric arteries. The sigmoid mesenteric artery supplies the caecum, ascending and most of the transverse colon. The inferior mesenteric artery supplies the remainder of the colon and the proximal part of the rectum. The distal of the rectum and the anus are supplied by branches from the internal iliac arteries. Venous drainage is mainly by the superior and inferior mesenteric veins which drain blood from the parts supplied by arteries of the same names. These veins join the splenic and gastric veins to form the portal vein. Veins draining the distal part of the rectum and the anus join the internal iliac veins.

COLONIC MICROFLORA

The slow movement of material through the colon allows a large microbial population to thrive there. Over 400 distinct bacterial species have been found, 20-30% of which are of the genus bacteroids. Most

of these isolated bacteria are anaerobic in nature; a small number of fungi are also present. The rate of microbial growth is greatest in the proximal areas because of high concentration of energy source. The principal sources of nutrition for the colonic microorganisms are carbohydrates are arriving in intestinal chime. The carbohydrates are degraded by the action of polysaccharides and glycosidase enzymes and the ultimate products of fermentation are short chain fatty acids, carbon dioxide, hydrogen, methane and hydrogen sulphide. In the proximal regions of the colon, carbohydrate fermentation predominates and results in a relatively low pH. In the distal regions, there is little carbohydrate fermentation, resulting in a higher pH. The bacteria within the colon are predominantly anaerobic and there is a low redox potential (reducing environment).

REGION OF GASTROINTESTINAL TRACT	CHARACTERISTICS
Large intestine	**Length in cm**
Caecum	6-7
Ascending colon	20
Transverse colon	45
Descending colon	30
Sigmoid colon	40
Rectum	12
Anal canal	3
Large intestine	**Intestinal diameter (cm)**
pH of Caecum and colon	5.5-7
Rectum	7
Colon	**Redox potential**
Right	-415
Mid	-400
Left	-380

FUNCTIONS

The major function of the colon is the consolidation of the intestinal contents into faeces by the absorption of water, and electrolytes and to store the faeces until excretion. The absorption capacity is very high; each day about 2000 ml of fluid enters the colon through the ileocaecal valve from which more than 90% of the fluid is absorbed. In the healthy human colon, sodium and chloride ions are usually secreted. On average, it has been estimated that colon contains only about 220g of wet material (Sarasija et al., 2000)

CANCER

Cancer is a group of more than 100 different diseases. They affect the body's basic unit, the cell. Cancer occurs when cells become abnormal and divide without control or order. Like all other organs of the body, the colon and rectum are made up of many types of cells. Normally, cells divide to produce more cells only when the body needs them. This orderly process helps keep us healthy.

If cells keep dividing when new cells are not needed, a mass of tissue forms. This mass of extra tissue, called a growth or tumor, can be benign or malignant.

Benign tumors are not cancer. They can usually be removed and, in most cases, they do not come back. Most important, cells from benign tumors do not spread to other parts of the body. Benign tumors are rarely a threat to life.

Malignant tumors are cancer. Cancer cells can invade and damage tissues and organs near the tumor. Also, cancer cells can break away from a malignant tumor and enter the bloodstream or lymphatic system. This is how cancer spreads from the original (primary) tumor to form new tumors in other parts of the body. The spread of cancer is called metastasis.

When cancer spreads to another part of the body, the new tumor has the same kind of abnormal cells and the same name as the primary tumor. For example, if colon cancer spreads to the liver, the cancer cells in the liver are colon cancer cells. The disease is metastatic colon cancer (it is not liver cancer).

COLON CANCER

Globally, cancer of the colon and rectum is the third leading cause of cancer in males and the fourth leading cause of cancer in females. The frequency of colorectal cancer varies around the world. It is common in the Western world and is rare in Asia and Africa. In countries where the people have adopted western diets, the incidence of colorectal cancer is increasing.

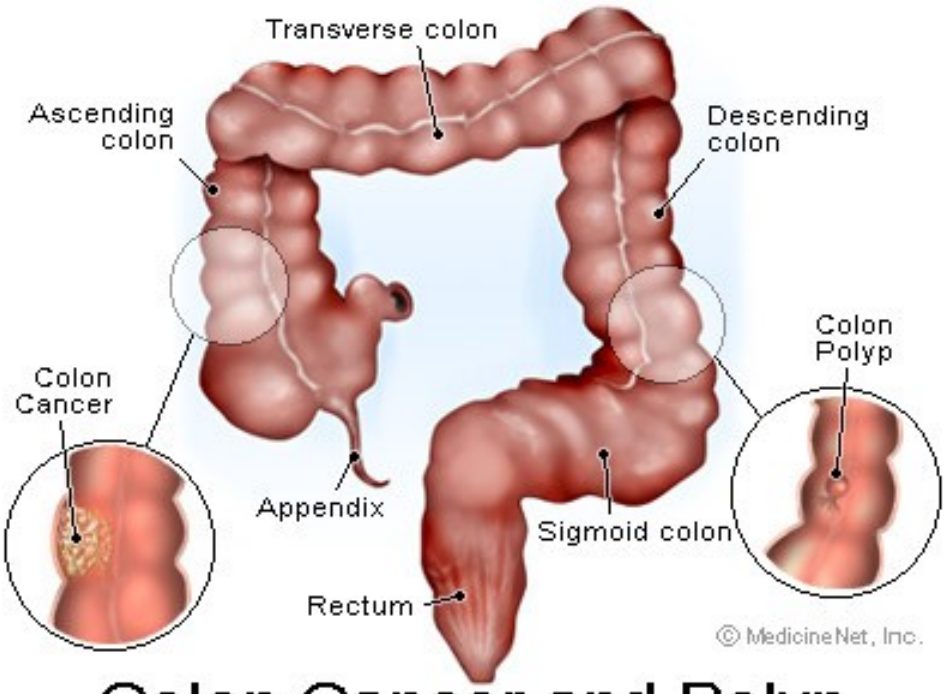
Colorectal cancer, also called colon cancer or large bowel cancer, includes cancerous growths in the colon, rectum and appendix With 655,000 deaths worldwide per year, it is the third most common form of cancer and the second leading cause of cancer-related death in the Western world. Many colorectal cancers are thought to arise from adenomatous polyps in the colon. These mushroom-like growths are usually benign, but some may develop into cancer over time. The majority of the time, the diagnosis of localized colon cancer is through colonoscopy. Therapy is usually through surgery, which in many cases is followed by chemotherapy.

The colon is the part of the digestive system where the waste material is stored. The rectum is the end of the colon adjacent to the anus. Together, they form a long, muscular tube called the large intestine (also known as the large bowel). Tumors of the colon and rectum are growths arising from the inner wall of the large intestine. Benign tumors of the large intestine are called polyps. Malignant tumors of the large intestine are called cancers.

Benign polyps do not invade nearby tissue or spread to other parts of the body. Benign polyps can be easily removed during colonoscopy and are not life-threatening. If benign polyps are not removed from the large intestine, they can become malignant (cancerous) over time.

Most of the cancers of the large intestine are believed to have developed from polyps. Cancer of the colon and rectum (also referred to as colorectal cancer) can invade and damage adjacent tissues and organs. Cancer cells can also break away and spread to other parts of the body (such as liver and lung) where new tumors form. The spread of colon cancer to distant organs is called metastasis of the colon cancer. Once metastasis has occurred in colorectal cancer, a complete cure of the cancer is unlikely.

Fig. 2 Colon Cancer



Colon Cancer and Polyp

SYMPTOMS

The first symptoms of colon cancer are usually vague, like bleeding, weight loss, and fatigue (tiredness). Local (bowel) symptoms are rare until the tumor has grown to a large size. Generally, the nearer the tumor is to the anus, the more bowel symptoms there will be.

Symptoms and signs are divided into local, constitutional and metastatic

LOCAL SYMPTOMS

- Change in bowel habits
    - Change in frequency of movements (constipation/or diarrhoea)
    - Feeling of incomplete defecation (tenesmus) and reduction in diameter of stool, both characteristic of rectal cancer,
    - Change in the appearance of stools:
        - Bloody stools or rectal bleeding
        - Stools with mucus
        - Black, tar-like stool (melena), more likely related to upper gastrointestinal e.g. stomach or duodenal disease
- Bowel obstruction causing bowel pain, bloating and vomiting of stool-like material.
- A tumor in the abdomen, felt by patients or their doctors.
- Symptoms related to invasion by the cancer of the bladder causing hematuria (blood in the urine) or pneumaturia (air in the urine), or invasion of the vagina causing maloderous vaginal discharge. These are late events, indicative of a large tumor.

CONSTITUTIONAL (SYSTEMIC) SYMPTOMS

- Unexplained weight loss is a worrying symptom caused by lack of appetite and systemic effects of a malignant growth. However, weight loss is not as much a feature of colorectal cancer as it is of other cancers (e.g. oesophageal carcinoma).
- Anemia, causing dizziness, fatigue and palpitations. Clinically, there will be pallor and blood tests will confirm the low hemoglobin level.

METASTATIC SYMPTOMS

- Liver metastases, causing:
  - Jaundice
  - Pain in the abdomen, more often the upper part of epigastrium or right side of the abdomen
  - liver enlargement, usually felt by a doctor
- Blood clots in the veins and arteries, a paraneoplastic syndrome related to hypercoagulability of the blood (the blood is "thickened").

TREATMENT

The treatment depends on the staging of the cancer. When colorectal cancer is caught at early stages (with little spread) it can be curable. However when it is detected at later stages (when distant metastases are present) it is less likely to be curable.

Surgery remains the primary treatment while chemotherapy and/or radiotherapy may be recommended depending on the individual patient's staging and other medical factors.

SURGERY

Surgeries can be categorised into curative, palliative, bypass, fecal diversion, or open-and-close.

Curative Surgical treatment can be offered if the tumor is localized.

- Very early cancer that develops within a polyp can often be cured by removing the polyp (i.e., polypectomy) at the time of colonoscopy.
- In colon cancer, a more advanced tumor typically requires surgical removal of the section of colon containing the tumor with sufficient margins, and radical en-bloc resection of mesentery and lymph nodes to reduce local recurrence (i.e., colectomy). If possible, the remaining parts of colon are anastomosed together to create a functioning colon. In cases when anastomosis is not possible, a stoma (artificial orifice) is created.
- Curative surgery on rectal cancer includes total mesorectal excision (lower anterior resection) or abdominoperineal excision.

In case of multiple metastases, palliative (non curative) resection of the primary tumor is still offered in order to reduce further morbidity caused by tumor bleeding, invasion, and its catabolic effect. Surgical removal of isolated liver

metastases is, however, common and may be curative in selected patients; improved chemotherapy has increased the number of patients who are offered surgical removal of isolated liver metastases.

If the tumor invaded into adjacent vital structures which makes excision technically difficult, the surgeons may prefer to bypass the tumor (ileotransverse bypass) or to do a proximal fecal diversion through a stoma.

The worst case would be an open-and-close surgery, when surgeons find the tumor unresectable and the small bowel involved; any more procedures would do more harm than good to the patient. This is uncommon with the advent of laparoscopy and better radiological imaging. Most of these cases formerly subjected to "open and close" procedures are now diagnosed in advance and surgery avoided.

Laparoscopic-assisted colectomy is a minimally-invasive technique that can reduce the size of the incision and may reduce post-operative pain.

As with any surgical procedure, colorectal surgery may result in complications including

- wound infection, dehiscence (bursting of wound) or hernia
- anastomosis breakdown, leading to abscess or fistula formation, and/or peritonitis
- bleeding with or without hematoma formation
- adhesions resulting in bowel obstruction (especially small bowel)
- adjacent organ injury; most commonly to the small intestine, ureters, spleen, or bladder
- cardiorespiratory complications such as myocardial infarction, pneumonia, arrythmia, pulmonary embolism etc

CHEMOTHERAPY

Chemotherapy is used to reduce the likelihood of metastasis developing, shrink tumor size, or slow tumor growth. Chemotherapy is often applied after surgery (adjuvant), before surgery (neo-adjuvant), or as the primary therapy (palliative). The treatments listed here have been shown in clinical trials to improve survival and/or reduce mortality rate and have been approved for use by the US Food and Drug Administration. In colon cancer, chemotherapy after surgery is usually only given if the cancer has spread to the lymph nodes (Stage III).

- Adjuvant (after surgery) chemotherapy. One regimen involves the combination of infusional 5-Fluorouracil leucovorin, and oxaliplatin (FOLFOX)
    - 5-fluorouracil (5-FU) or Capecitabine (Xeloda)
    - Leucovorin (LV, Folinic Acid)
    - Oxaliplatin (Eloxatin)
- Chemotherapy for metastatic disease. Commonly used first line chemotherapy regimens involve the combination of infusional 5-Fluorouracil, leucovorin and oxaliplatin (FOLFOX with bevacizumab or infusional 5-Fluorouracil, leucovorin, and irinotecan (FOLFIRI) with bevacizumab.
    - 5-Fluorouracil (5-FU) or Capecitabine
    - UFT or Tegafur-uracil
    - Leucovorin (LV, Folinic Acid)

- Irinotecan (Camptosar)
- Oxaliplatin (Eloxatin)
- Bevacizumab (Avastin)
- Cetuximab (Erbitux)
- Panitumumab (Vectibix)

- In clinical trials for treated/untreated metastatic disease.
  - Bortezomib (Velcade)
  - Oblimersen (Genasense, G3139)
  - Gefitinib and Erlotinib (Tarceva)
  - Topotecan (Hycamtin)

RADIATION THERAPY

Radiotherapy is not used routinely in colon cancer, as it could lead to radiation enteritis, and it is difficult to target specific portions of the colon. It is more common for radiation to be used in rectal cancer, since the rectum does not move as much as the colon and is thus easier to target. Indications include:

COLON CANCER

- Pain relief and palliation - targeted at metastatic tumor deposits if they compress vital structures and/or cause pain

RECTAL CANCER

- neoadjuvant - given before surgery in patients with tumors that extend outside the rectum or have spread to regional lymph nodes, in order to decrease the risk of

recurrence following surgery or to allow for less invasive surgical approaches (such as a low anterior resection instead of an abdomino-perineal resection).

- adjuvant - where a tumor perforates the rectum or involves regional lymph nodes palliative - to decrease the tumor burden in order to relieve or prevent symptoms.

Sometimes chemotherapy agents are used to increase the effectiveness of radiation by sensitizing tumor cells if present.

IMMUNOTHERAPY

Bacillus Calmette-Guérin (BCG) is being investigated as an adjuvant mixed with autologous tumor cells in immunotherapy for colorectal cancer.

VACCINE

In November 2006, it was announced that a vaccine had been developed and tested with very promising results. The new vaccine, called TroVax works in a totally different way to existing treatments by harnessing the patient's own immune system to fight the disease. Experts say this suggests that gene therapy vaccines could prove an effective treatment for a whole range of cancers. Oxford Bio Medica is a British spin-out from Oxford University specialising in the development of gene-based treatments Phase III trials are underway for renal cancers and planned for colon cancers.

APPROACHES TO COLONIC DRUG DELIVERY VIA THE ORAL ROUTE

The challenge of targeting drugs specifically to the colonic region of the gastrointestinal tract is one that has been embraced by scientists over the past two decades. The colon has recently become accepted as an increasingly important site for drug delivery. Research interest in the area of colonic drug delivery has been fuelled by the need to better treat pathologies of the colon that range in seriousness from constipation and diarrhoea to the debilitating inflammatory bowel diseases (ulcerative colitis and Crohn's disease) through to colon carcinoma, the third most prevalent form of cancer in both men and women. Targeted drug delivery to the colon would therefore ensure direct treatment at the disease site, lower dosing and a reduction in systemic side effects. Aside from local treatment, the colon can also be utilized as a portal for the entry of drugs into the blood stream for the purpose of systemic therapy. Drugs that are degraded and /or poorly absorbed in the upper gut may be preferentially absorbed from the colon because of the lower levels of luminal and mucosal digestive enzymes, as compared with the small intestine .Further more, colonic drug delivery may also be used as a means of achieving chronotherapy

for diseases that are sensitive to circulation rhythms, such as asthma and arthritis.

Many proteins and peptide drugs like insulin cannot be administered through the oral route because of their degradation by the digestive enzymes of the stomach and the small intestine. Delivery of the drugs to the systemic circulation through colonic absorption represents a novel mode of introducing peptides and protein drug molecules and drugs that absorb poorly from the upper gastrointestinal tract as the colon lacks various digestive enzymes present in the upper GIT. The drug targeting through colon not only reduces the dose to be administered but also reduces the incidence of possible adverse effects associated with these chemotherapeutic agents (Sinha et al., 2001).

The various strategies for targeting orally administered drugs to the colon include covalent linkage of a drug with a carrier, coating with pH-sensitive polymers, formulation of timed released systems, exploitation of carriers that are degraded specifically by colonic bacteria, bioadhesive systems and osmotic controlled drug delivery systems. Various prodrugs (sulfasalazine, ipsalazine, balsalazine and olsalazine) have been developed that are aimed to deliver 5-Amino salicylic acid (5-ASA) for localized chemotherapy of inflammatory

bowel disease (IBD). Microbially degradable polymers especially azo crosslinked polymers have been investigated for use in targeting of drugs to colon. Certain plant polysaccharides such as amylose, inulin, pectin and guar gum remains unaffected in the presence of gastrointestinal enzymes and pave the way for the formulation of colon targeted drug delivery systems. The concept of using pH as a trigger to release a drug in the colon is based on the pH conditions that vary continuously down the gastrointestinal tract. Times dependent drug delivery systems have been developed that are based on the principle to prevent release of drug until 3-4 h after leaving the stomach. Redox sensitive polymers and bioadhesive systems have also been exploited to deliver the drugs into the colon.

REVIEW OF LITERATURE

Hang Lin *et al.*, (2008) prepared of 5-Fluorouracil loaded polylactide-co-glycolide-co-methoxy poly (ethylene glycol) (PLGA-mPEG) nanoparticles via high speed shearing 5-Fluorouracil (5-Fu) loaded nanoparticles (NPs) were prepared by a high speed shearing double emulsion method with polylactide-co-glycolide-co-methoxy poly (ethylene glycol) (PLGA-mPEG) as loading material. The prepared NPs possess a negative zeta potential and their loading efficiency is about 15% (mass fraction). The result of *in vitro* release shows that the release behavior of 5-Fu from NP's is coincident with zero-level release from the second day.

Rakesh Patel, (2008) reviewed nanotechnology is the synergy of mechanical, material sciences, microelectronics, electrical, chemical and biological screening. Nanotechnologies are the design, characterization, production and application of structures, devices and systems by controlling shape and size at nanometer scale. This systemic review highlights classifications, preparation techniques, characterization methods, applications, health implications and clinical aspects of nanoparticles.

Ravi et *al.*, (2008) developed a novel colon targeted tablet formulation using pectin as carrier and diltiazem HCl and indomethacin as model drugs. The tablets were coated with inulin followed by shellac and were evaluated for average weight, hardness and coat thickness. *In vitro* release studies for prepared tablets were carried out for 2 h in pH 1.2 HCl buffer, 3 h in pH 7.4 phosphate buffer and 6 h in

simulated colonic fluid. The drug release from the coated systems was monitored using UV/V is spectroscopy. *In vitro* studies revealed that the tablets coated with inulin and shellac have limited the drug release in stomach and small intestinal environment and released maximum amount of drug in the colonic environment. The study revealed that polysaccharides as carriers and inulin and shellac as a coating material can be used effectively for colon targeting of both water soluble and insoluble drugs.

Su Li *et al.*, (2008) studied the pharmacokinetic characteristics and anticancer effects of 5-Fluorouracil loaded nanoparticles. It is expected that, prolonged circulation of anticancer drugs will increase their anticancer activity while decreasing their toxic side effects. The purpose of this study was to prepare 5-fluorouracil (5-Fu) loaded block copolymers, with poly(γ-benzyl-L-glutamate) (PBLG) as the hydrophobic block and poly(ethylene glycol) (PEG) as the hydrophilic block, and then examine the 5-Fu release characteristics, pharmacokinetics, and anticancer effects of this novel compound 5-Fu loaded PEG-PBLG (5-Fu/PEG-PBLG) nanoparticles were prepared by dialysis and then scanning electron microscopy (SEM) and transmission electron microscopy (TEM) were used to observe the shape and size of the nanoparticles, and ultraviolet spectrophotometry was used to evaluate the 5-Fu *in vitro* release characteristics. The pharmacokinetic parameters of 5-Fu/PEG-PBLG nanoparticles in rabbit plasma were determined by measuring the 5-Fu by high-performance

liquid chromatography (HPLC). To study *in vivo* effects, LoVo cells (human colon cancer cell line) or Tca8113 cells (human oral squamous cell carcinoma cell line) were implanted in BALB/c nude mice that were subsequently treated with 5-Fu or 5-Fu/PEG-PBLG nanospheres. In this model system, 5-Fu/PEG-PBLG nanoparticles changed the pharmacokinetic behavior of 5-Fu, thus increasing its anticancer activity. 5-Fluorouracil loaded nanoparticles have potential as a novel anticancer drug that may have useful clinical applications.

Xueming Li *et al.*, (2008) investigated PLGA nanoparticles for the oral delivery of 5-Fluorouracil using high pressure homogenization-emulsification as the preparation method and *in vitro/in vivo* to incorporate the hydrophilic anti-cancer drug 5-Fluorouracil (5-Fu) into poly(lactide-co-glycolide) (PLGA) nanoparticles (NP) to improve the oral bioavailability. Owing to the high solubility of 5-Fu in basic water, the water-in-oil-in-water (w/o/w) emulsification process has been chosen as one of the most appropriate method for the encapsulation of 5-Fu, and the ammonia solution was used as the inner aqueous phase solvent to increase the solubility of 5-Fu. In order to reach submicron size as well as increasing the grade of monodispersity compared to previous preparation techniques, prepared 5-Fu loaded PLGA-NP by a high-pressure emulsification-solvent evaporation process. The PLGA-

NPs were characterized with respect to their morphology, particle size, size distribution, 5-Fu encapsulation efficiency, *in vitro* and *in vivo* studies in rats. *In vitro* release of 5-Fu from nanoparticles appeared to have two components with an initial rapid release due to the surface associated drug and followed by a slower exponential release of 5-Fu, which was dissolved in the core. The *in vivo* research was studied in male Sprague-Dawley rats after an oral 5-Fu dose of 45 mg/kg. Single oral administration of 5-Fu loaded PLGA-NP to rats produced bioavailability, which was statistically higher than 5-Fu solution as negative control and the MRT (mean residence time) of 5-Fu loaded PLGA-NP was significantly ($p < 0.05$) modified. Thus, it is possible to design a controlled drug delivery system for oral 5-Fu delivery, improving therapy efficiency by possible reduction of time intervals between peroral administrations and reduction of local gastrointestinal side effects.

Harikrishna Devalapally *et al.*, (2007) reviewed the role of nanotechnology in pharmaceutical product development. Nanoparticle technology initially arose to address solubility and permeability problems in drug development. Advances in the area of nanoparticulate drug delivery systems have allowed some of the molecules in the market to achieve desirable pharmacokinetic

properties and reducing the unwanted toxicity, improving patient compliance, and clinical outcomes. In the development of NMEs can be achieved by using specific types of nanosystems in the preformulation development to obtain a balance between chemistry, pharmacology, and pharmacokinetics of drug.

Shinji Sakuma *et al.*, (2006) reviewed the development of a dosage form that improves the absorption of peptide and protein drugs via the gastrointestinal tract are one of the greatest challenges in the pharmaceutical field. Many researchers have taken up the challenge, using approaches including mucoadhesive drug delivery, colon delivery, particulate drug delivery such as nanoparticles, microcapsules, liposomes, emulsions, micelles, and so on. The objective of this article is to provide the reader with outlines of novel nanoparticle technologies for oral peptide delivery based on polymer chemistry. The physicochemical properties of nanoparticles and their behavior on exposure to physiological media are greatly dominated by their chemical structures and surface characteristics.

Fu-de Cui *et al.*, (2006) investigated the preparation of PLGA nanoparticles (PNP) and PLGA-Hp55 nanoparticles (PHNP) as potential drug carriers for oral insulin delivery. The nanoparticles were prepared by a modified emulsion solvent diffusion method in water, and

their physicochemical characteristics, drug release *in vitro* and hypoglycemic effects in diabetic rats were evaluated. The particle sizes of the PNP and PHNP were 150 ± 17 and 169 ± 16 nm, respectively, and the drug recoveries of the nanoparticles were 50.30 ± 3.1 and 65.41 ± 2.3%, respectively. The initial release of insulin from the nanoparticles in simulated gastric fluid over 1 h was 50.46 ± 6.31 and 19.77 ± 3.15%, respectively. The relative bioavailability of PNP and PHNP compared with subcutaneous (s.c.) injection (1 IU/kg) in diabetic rats was 3.68 ± 0.29 and 6.27 ± 0.42%, respectively. The result revealed that, the use of insulin-loaded PHNP was an effective method of reducing serum glucose levels.

Paula De Angelis *et al.*, (2006) cellular response to 5-Fluorouracil (5-Fu) in 5-Fu-resistant colon cancer cell lines during treatment and recovery gene expression data suggested that, altered regulation of nucleotide metabolism, amino acid metabolism, cytoskeleton organization, transport, and oxygen metabolism may underlie the differential resistance to 5-Fu seen in these cell lines.

Tuleu *et al.*, (2006) investigated seven healthy male volunteers received on three separate occasions, an uncoated or amylose-ethylcellulose-coated hydroxypropylmethylcellulose capsule containing 4-aminosalicylic acid Na (550 mg), or an *intravenous* injection of 4-aminosalicylic acid Na (135 mg). The capsules were radiolabelled with ^{99m}Tc to

allow their positions in the gastrointestinal tract to be followed using a gamma camera. Plasma and urine samples were collected and assayed for 4-Aminosalicylic acid and metabolite concentrations. The uncoated capsules broke down within 10 min in the stomach, allowing rapid and complete absorption of the drug. The coated capsules remained intact in the upper gastrointestinal tract, and had a median gastric emptying time of 61 min (interquartile range, 77 min) and a median colon arrival time of 363 min (interquartile range, 185 min). For the coated capsules, only the metabolite was detected in the plasma and/or urine after the capsules had reached the colon. The specific coating protected the drug until the capsule reached the colon, where 4-Aminosalicylic acid was slowly released and absorbed. Thus, such a formulation has the potential for use in the treatment of inflammatory bowel disease.

Ziyaur Rahman *et al.*, (2006) prepared and evaluated the colon-specific microspheres of 5-Fluorouracil for the treatment of colon cancer. Core microspheres of alginate were prepared by the modified emulsification method in liquid paraffin and by cross-linking with calcium chloride. The core microspheres were coated with Eudragit S-100 by the solvent evaporation technique to prevent drug release in the stomach and small intestine. The microspheres were characterized by shape, size, surface morphology, size distribution, incorporation efficiency, and *in vitro* drug release studies. The outer surfaces of the core and coated microspheres, which were spherical in shape, were rough and smooth, respectively. The size of the core microspheres ranged from 22 to 55 µm, and the size of the coated microspheres ranged from 103 to 185 µm. The core microspheres sustained the drug release for 10 hours. The release studies of coated microspheres were performed in a pH progression medium mimicking the conditions of the gastrointestinal tract. Release was sustained for up to 20 hours in formulations with core microspheres to a Eudragit

S-100 coat ratio of 1:7, and there were no changes in the size, shape, drug content, differential scanning calorimetry thermogram, and *in vitro* drug release after storage at 40°C/75% relative humidity for 6 months.

Krishna *et al.*, (2005) reviewed that nanoparticles are one of the novel colloidal drug delivery systems that hold great promise for reaching the goal of controlled drug delivery as well as site-specific delivery. The present review highlights that several carriers used in the preparation of NPs method of preparation. Various pharmaceutical considerations involved interaction of NPs with blood components and cell membranes, the physiochemical characterization of NPs and their therapeutic applications in the field of medicine. Nanoparticles are used for parentral, oral, ocular and transdermal applications as well as used in cosmetics and hair care technologies, sustained release formulations and as a carrier for radio nucleotides in nuclear medicine.

Asuman Bozkir *et al.*, (2005) prepared and evaluated an orthogonal experimental design to optimize the formulation of 5-Fluorouracil (5-Fu) loaded poly (D-L-Lactide-co-glycolide) (PLGA) nanoparticles (5-Fu-NP) by a nanoprecipitation-solvent displacement technique. The type of surfactant, amount of acetone and molecular weight of the polymer with three levels of each factor were selected and arranged in an $L_{18}(3^5)$ orthogonal experimental table. From the statistical analysis of the data, polynomial equations were generated. Optimized formulations have the particle size ranging from 160 to 250 nm. Smallest nanoparticles (161 ± 1.22 nm) were obtained using Resomer PLGA 755 and pluronic

F-68 with 10 ml acetone amount. Under these conditions the 5-Fu entrapment percentage was maximum 78.30%, suggesting 5-Fu might be entrapped and adsorbed on the nanoparticle surface. *In vitro* release of three formulations with maximum drug entrapment efficiency and minimum particle size, were also investigated by release kinetics. According to the determined coefficients, release data fit to Higuchi's diffusion kinetics. The *in vitro* release of 5-Fu-NP in phosphate buffered saline (PBS, pH 7.4) is suggested to be controlled by a combination of diffusion with slow and gradual erosion of the particles. Also, the antimicrobial activity was observed even on the end of seventh day with all formulations.

Ugo Bilati *et al.*, (2005) investigated the entrapment of 3 different model proteins (tetanus toxoid, lysozyme, and insulin) into poly (D,L-lactic acid) and poly(D,L-lactic-*co*-glycolic acid) nanoparticles and to address process-related stability issues. For that purpose, a modified nanoprecipitation method as well as 2 emulsion-based encapsulation techniques (i.e., a solid-in oil-in water (s/o/w) and a double emulsion ($w_1/o/w_2$) method) were used. The main modification of nanoprecipitation involved the use of a wide range of miscible organic solvents such as dimethylsulfoxide and ethanol instead of the common acetone and water. The results obtained showed that tetanus toxoid and lysozyme were efficiently incorporated by the double emulsion procedure when ethyl acetate was used as solvent (>80% entrapment efficiency), The nanoprecipitation method led to a homogenous population of small nanoparticles (with size ranging from

~130 to 560 nm) and in some cases also improved experimental drug loadings, especially for lysozyme (entrapment efficiency >90%). With respect to protein stability, the duration and intensity of sonication were not a concern for tetanus toxoid, which retained more than 95% of its antigenicity after treatment for 1 minute. Only a high methylene chloride:water ratio was shown to slightly decrease toxoid antigenicity. Finally, no more than 3.3% of A21 desamido insulin and only traces of covalent insulin dimer were detected in nanoparticles. In conclusion, both the double emulsion and nanoprecipitation methods allowed efficient protein encapsulation. MALDI-TOF MS allowed accurate drug content determination. The manufacturing processes evaluated did not damage the primary structure of insulin.

Peter Watts *et al.*, (2005) designed a technology for site-specific delivery of drugs in the gastrointestinal (GI) tract and, in particular, targeted release into the colonic region. A key area of application is the delivery of therapeutic agents for local treatment of lower GI diseases. The technology is based on the application of pH-sensitive coatings onto injection-moulded starch capsules. An extensive body of clinical data has been generated showing reliable *in vivo* performance of the capsules. In γ-scintigraphy studies around 90% of TARGIT capsules (n=84) delivered their contents to the target site of the terminal ileum and colon. TARGIT-based products are in active clinical development for the treatment of conditions including inflammatory bowel diseases.

Shantha *et al.*, (2005) reviewed colon-targeted delivery of bioactives has recently gained importance in addressing specific needs in the therapy of colon-based diseases. Many approaches have been attempted for the development of colon-specific delivery systems, with not much success in the past. Recent research into the utilization of the metabolic activity and the colonic microenvironment in the lower gastrointestinal tract has attained great value in the design of novel colon-targeted delivery systems based on natural biodegradable polymers. In the current article, special emphasis has been placed on polysaccharide systems, with minimal chemical modification, that have been exploited for colon targeting. These polysaccharide based encapsulation and targeted delivery systems are envisaged to have an immense potential for the development of food/nutraceutical formulations for colon-based diseases, including colorectal cancer.

Ugo Bilati *et al.*, (2004) investigated formulation and process modifications to improve the versatility of the nanoprecipitation technique, particularly with respect to the encapsulation of hydrophilic drugs (e.g. proteins). More specifically, the principal objective was to explore the influence of such modifications on nanoparticle size. Selected parameters of the nanoprecipitation method, such as the solvent and the non-solvent nature, the solvent/non-solvent volume ratio and the polymer concentration, were varied so as to obtain polymeric nano-carriers. The feasibility of such a modified method was assessed and resulting unloaded nanoparticles were characterized with respect to their size and shape. It was shown that the mean particle size was closely dependent on the type of non-solvent selected. When alcohols were used, the final mean size increased in the sequence: methanol < ethanol < propanol. Surfactants added to the dispersing medium were usually unnecessary for final suspension stabilization. Changing the solvent/non-solvent volume ratio was also not a determinant factor for nanoparticle formation and their

final characteristics, provided that the final mixture itself did not become a solvent for the polymer. A too high polymer concentration in the solvent, however, prevented nanoparticle formation. Both poly (lactic acid) (PLA) and poly (D-L-lactic-co-glycolic acid) (PLGA) could be used by accurately choosing the polymer solvent and in this respect, some non-toxic solvents with different dielectric constants were selected. The nanoparticles obtained ranged from about 85–560 nm in size. The nanoparticle recovery step however needs further improvements, since bridges between particles which cause flocculation could be observed. The results revealed that the nanoprecipitation technique is more versatile and flexible than previously thought and that a wide range of parameters can be modified.

Krishnaiah *et al.*, (2002) conducted *in vivo* evaluation of orally administered guar gum-based colon-targeted tablet formulations of celecoxib (colon-targeted tablet-20 or colon-targeted tablet-30) as compared with an immediate release capsule in 15 human volunteers. Blood samples were obtained at different time intervals and the plasma concentration of celecoxib was estimated by reversed phase HPLC. The immediate release capsules of celecoxib might have disintegrated very fast in GI tract and absorbed quickly from stomach and small intestine thereby producing peak plasma concentration (C_{max} of 478±57 ng/ml) within 3.8±0.1 h (T_{max}). Though celecoxib could be seen in plasma after oral administration of colon-targeted tablet-20 or colon-targeted tablet-30 between 1 and 2 h, low levels of drug were observed up to 8 h resulting in peak concentration (C_{max}) of 78±6 ng/ml or 88±15 ng/ml at 10.5±1.9 h or 13.5±1.4 h (T_{max}) respectively, whereas the immediate release capsules produced peak plasma concentration (C_{max}) of 478±57 ng/ml at 3.8±0.1 h (T_{max}). Colon-targeted tablets showed decreased $AUC_{0-\infty}$, C_{max} and absorption rate constant, prolonged absorption time (t_a) and increased $t_{1/2}$ in

comparison with the immediate release capsules. The results of the study indicated that the guar gum-based colon-targeted tablets of celecoxib did not release the drug significantly in stomach and small intestine, but delivered to the colon resulting in a slow absorption of the drug and making it available for local action in the colon.

Sinha *et al.*, (2001) reviewed polysaccharides in colon-specific drug delivery. Natural polysaccharides are now extensively used for the development of solid dosage forms for delivery of drug to the colon. The rationale for the development of a polysaccharide based delivery system for colon is the presence of large amounts of polysaccharidases in the human colon as the colon is inhabited by a large number and variety of bacteria which secrete many enzymes. E.g. D-galctosidase, amylase, pectinase, xylanase, dextranase etc. Various major approaches utilizing polysaccharides for colon-specific delivery are fermenting coating of the drug core, embedding of the drug biodegradable matrix and formulation of drug-saccharide conjugate (prodrugs). A large number of polysaccharides have already been studied for their potential as colon-specific drug carrier systems, such as chitosan, pectin, guar gum, inulin etc. Recent efforts and approaches exploiting these polysaccharides in colon-specific drug delivery are discussed.

Miller *et al.*, (2001) reviewed that the major problem of many newly developed pharmaceutical drugs is their poor solubility in water and simultaneously in organic media. To solve these problems formulation as nanosuspensions is an attractive alternative. During, the drug development process screening for an optimal formulation by homogenization is essential. Time and cost effective production in an initial phase of R&D can be conducted on lab scale by using the Micron Lab 40 in its discontinuous version. Reproducibility of small scale production parameters (particle size, size distribution, content of microparticles) was exemplary studied for the drug RMKP22.

Wen Juan Jia *et al.*, (2000) a biodegradable polyetherester copolymer (PCL/PEG/PCL, PCEC) as synthesized by ring-opening polymerization of caprolactone initiated by poly(ethylene glycol) (PEG). The PCEC nanoparticles were prepared by solvent diffusion method or w/o/w double emulsion method. The obtained particles' morphology was observed on scanning electron microscopy, and the particle size distribution was determined using Malvern laser particle sizer. Bovine serum albumin was used as the model water-soluble protein drug, which was successfully encapsulated in PCEC nanoparticles; the drug release behavior was studied in detail. The hydrolytic degradation behavior of the PCEC nanoparticles was also studied.

Sarasija *et al.*, (1999) reviewed that the colon specific delivery of drugs are of interest for the treatment of colonic diseases, so as to maximize the effectiveness of these drugs. Oral delivery of peptides and proteins are possible because colon provides a more friendly environment than the upper gastrointestinal tract. Colon is also rich in lymphoid tissue so uptake of antigens into the mast cells of the colonic mucosa produces rapid local production of antibodies thus helping in vaccine delivery. This review dealt with the anatomy and physiology of colon and various aspects of formulations by which colon targeting of drugs can be achieved.

Thirumala Govender *et al.*, (1999) investigated the nanoprecipitation technique for preparation of nanoparticles suffers the drawback of poor incorporation of water soluble drugs. The aim of this study was therefore to assess various formulation parameters to enhance the incorporation of a water soluble drug (procaine hydrochloride) into poly (D-L-Lactide-co-glycolide) (PLGA) nanoparticles prepared by this technique. Approaches investigated for drug incorporation efficiency enhancement included the influence of aqueous phase pH, replacement of procaine hydrochloride with procaine dihydrate and the inclusion of excipients: poly (D-L-lactide) (PLA) oligomers, poly (methyl methacrylate-co-methacrylic acid)

(PMMA–MA) or fatty acids into the formulation. The nanoparticles produced were submicron size (<210 nm) and of low polydispersity. It was found that an aqueous phase pH of 9.3, replacement of procaine hydrochloride with procaine dihydrate and the incorporation of PMMA–MA, lauric and caprylic acid into the formulation could enhance drug incorporation efficiency without the size, morphology and nanoparticle recovery being adversely influenced. For instance changing the aqueous phase pH from 5.8 to 9.3 increased nanoparticle recovery from 65.1 to 93.4%, drug content from 0.3 to 1.3% w/w and drug entrapment from 11 to 58.2%. However, the presence of high ratios of lauric acid and procaine dihydrate in the formulation adversely affected the morphology and size of the nanoparticles. Also, PLA oligomers were not considered a feasible approach since it decreased drug entrapment from 11 to 8.4% and nanoparticle recovery from 65.1 to 19.6%. Drug release from nanoparticles appears to consist of two components with an initial rapid release followed by a slower exponential stage. This study has demonstrated that formulation variables can be exploited in order to enhance the incorporation of a water soluble drug into PLGA nanoparticles by the nanoprecipitation technique.

David Quintanar-Guerrero *et al.*, (1998) reviewed the techniques available to prepare biodegradable nanoparticles from preformed polymers. Although there is abundant literature on this topic, only a few focuses on the thorough analysis of preparative procedures, In particular, four techniques are discussed in terms of their technological advantages and drawbacks: emulsification, evaporation, solvent displacement, salting out and emulsification diffusion. The proposed mechanism of nanoparticle formation for each technique is described from a physicochemical perspective. The effects of preparative variables on nanoparticle size and drug-entrapment efficiency are also discussed.

Shoba Rani *et al.*, (1998) reviewed the importance nanoparticles as drug delivery systems. Nanoparticles, one of the colloidal drug delivery systems, hold great promise for reaching the goal of controlled drug delivery as well as site specific delivery. This review presents various methods of preparation, characterization, stability, drug release and applications of nanoparticles. If appropriately investigated, nanoparticles may open new avenues in research and therapy.

Wilding *et al.*, (1998) investigated the scintigraphic evaluation of a new capsule-type colon specific drug delivery system in healthy volunteers to provide "proof of concept" for a novel capsule-type colonic delivery system (colon targeted delivery capsule). The lack of predictive *in vitro* or animal model leads to considerable time delays in colonic product development .The human data validates the design concept behind the release mechanism, in that capsule disintegration, and hence drug release, did not start until 5 h after gastric emptying irrespective of whether the product was administered to fasted or fed subjects. However, the potential for prolonged gastric residence for large enteric coated products intended for intestinal targeting was also observed. Overall, the study provides a focus for subsequent product development and highlights the role of scinitigraphy in dynamically visualizing the drug delivery process.

Takashi Ishibashi *et al.*, (1998) investigated a new capsule-type dosage form for colon-targeted delivery of drugs. The system was designed by imparting a timed-release function and a pH-sensing function to a hard gelatin capsule. The technical characteristics of the system are to contain an organic acid together with an active ingredient in a capsule coated with a three-layered film consisting of an acid-soluble polymer, a water-soluble polymer, and an enteric polymer. In order to find the suitable formulation, various formulation factors were investigated through a series of *in vitro* dissolution studies. As a result, it was found that: (1) various organic acids can be used for this system; (2) a predictable timed-release mechanism of a drug can be

attained by adjusting the thickness of the Eudragit E layer; and (3) the outer enteric coating with HPMC-AS provided acceptable acid-resistibility. All these results suggested that this approach can provide a useful and practical means for colon-targeted delivery of drugs.

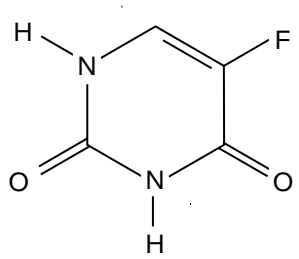
Van Den Mooter *et al.*, (1995) evaluated azo polymers based upon 2-hydroxyethyl methacrylate, methyl methacrylate, and methacrylic acid, and containing N,N'-bis [(methacryloyloxyethyl) oxy(carbonylamino)]azobenzene as azo aromatic agent *in vivo* as coatings for colon-specific drug delivery. The gastrointestinal absorption of theophylline from capsules coated with the azo polymers was examined in the proximal part of the small intestine and the caecum of male Wistar rats. The capsules were surgically inserted in the region of interest. The plasma concentration of the drug was higher when the capsules were inserted in the caecum as compared to the small intestine. The appearance of theophylline in the plasma when capsules were administered in the small intestine can be attributed to simple diffusion of the drug through the swollen polymer coating. Release and absorption from the caecum is the combined result of diffusion and degradation of the azo polymer coatings by bacterial azo reductase.

Proano *et al.*, (1990) used a noninvasive method to label the solid phase of contents in the unprepared human colon. 111In-labeled Amberlite pellets (0.5-1.8 mm dia) were placed in a gelatin capsule that was then coated with a pH-sensitive polymer (methacrylate). *In vitro*, the capsules disintegrated in simulated small bowel contents within
1-2 h; when ingested by healthy subjects, capsules released radiolabel in the distal ileum or proximal colon in 13 of 15 subjects. Transit of 111In-pellets through the unprepared colon could then be quantitated radioscintigraphically. Segmental transit was defined in the ascending (AC), transverse (TC), descending (DC), and

rectosigmoid (RS) colon. Radioactivity was also quantitated in stools. At 12 h, radioactivity was most obvious in the AC (59 +/- 11%, mean +/- SE) and the TC (21 +/- 6%); at 24 h, counts were distributed equally between AC, TC, and stools (P greater than 0.05); by 48 h, 56 +/- 11% counts had been excreted, although 30 +/- 10% remained in the TC. At 24 and 48 h, the amount in DC or RS was lower ($p < 0.05$) than in the TC or in stools. Emptying of the AC was characterized by an initial lag period, when no counts emptied into the TC, followed by a period of emptying that was approximately linear. Thus this simple approach is able to label contents in the healthy human colon. The ascending and transverse colon appear to be sites of storage of solid residue, whereas the left colon and rectosigmoid function mainly as conduits.

DRUG PROFILE

STRUCTURE OF 5-FLUOROURACIL



Category	:	A pyramidine antagonist, is an antimetabolite Antineoplastic agent
Chemical name	:	2, 4(1H, 3H)-pyrimidinedione
Synonyms	:	2, 4-Dihydroxy-5-fluropyrimidine 5-Fu; 5-Fluracil
Molecular weight	:	130.08
Molecular formula	:	$C_4H_3FN_2O_2$
Appearance	:	Solid
Color	:	White
Freezing / melting point	:	280-282° C
Decomposition temperature	:	80° C

Solubility	:	Sparingly soluble in water; slightly soluble in ethanol (95%); practically insoluble in chloroform and in ether
Conditions to avoid	:	Light
Chemical stability	:	Stable under normal temperatures and pressure.
Trade names	:	NSC-19-893; Ro-Efudex; Fluroplex; fluorouracil

Absorption spectroscopy

The UV absorption spectrum shows a maximum at 265-266 nm (epsilon=7.070) and a minimum at 232 nm (in acetate buffer, pH 4.7).This, as well as IR, NMR (proton and 19 F), and florescence spectral data (λ_{max} exc =315, λ_{max} em =391)

Adverse effects

- **CNS**: Lethargy, malaise, weakness, euphoria, acute cerebellar syndrome, photophobia, lacrimation, decreased vision, nystagmus, diplopia

- **GI**: Diarrhea, anorexia, nausea, vomiting, cramps, enteritis, duodenal ulcer, duodenitis, gastritis, glossitis, stomatitis, pharyngitis, esophagopharyngitis
- **CV**: Myocardial ischemia, angina
- **Hematologic**: Leukopenia, thrombocytopenia, elevations in alkaline phosphatase, serum transaminase, serum bilirubin, lactic dehydrogenase
- **Dermatologic**: Alopecia, dermatitis, maculopapular rash, photosensitivity, nail changes including nail loss, dry skin, fissures
- **Other**: Fever, Epistaxis

5-Fluorouracil (5-Fu), a pyrimidine analog, has a stable fluorine atom in place of a hydrogen atom at position 5 of the uracil ring. The fluorine interferes with the conversion of deoxyuridylic acid, thus depriving the cell of one of the essential precursors for DNA synthesis. 5-Fu is employed primarily in the treatment of solid growing solid tumors (for example, colorectal, breast, ovarian, pancreatic, and gastric carcinomas). Adjuvant therapy with levamisole - a veterinary anthelmintic agent - improves the survival of some patients with colon cancer. When applied topically, 5-Fu is also effective for the treatment of superficial basal cell carcinomas.

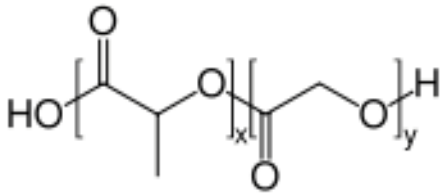
1. **Mechanism of action**: 5-Fu has a good antineoplastic activity. It enters the cell through a carrier - mediated transport system and is converted to the corresponding deoxynucleotide (5-FdUMP), which competes with deoxyuridine monophosphate for thymidylate synthase. 5FdUMP acts as apseusubstrate and is trapped with the enzyme and its N^5, N^{10}–methylene tetrahydrofolic acid (leucovorin) coenzyme in a ternary complex that cannot proceed to products. DNA synthesis decreases due to lack of thymidine, leading to imbalanced cell growth and cell death. 5-Fu is also incorporated into RNA, and low levels have been detected in DNA. In the later case, a glycosylase excises the 5-Fu damaging the DNA.

2. **Pharmacokinetics:** .Because of its severe toxicity to the GI tract, 5-Fu is given in the case of skin cancer, topically. The drug penetrates well into all tissues, including the CNS. 5-Fu is rapidly metabolized in the liver, lung and kidney; it is eventually converted to CO_2, which is exhaled. The dose of 5-Fu must be adjusted in the case of hepatic function.(Tripathi et al., 2008 and Mary J.Mycek et al., 1997)

POLYMER PROFILE

1. POLY (D, L-LACTIDE-CO-GLYCOLIDE) PLGA

Structure



PLGA is a *copolymer* which is used in a host of *Food and Drug Administration* (FDA) approved therapeutic devices, owing to its *biodegradability* and *biocompatibility*. PLGA is synthesized by means of random ring-opening co-polymerization of two different *monomers*, the cyclic dimers (1,4-dioxane-2,5-diones) of *glycolic acid* and *lactic acid*.

Depending on the ratio of lactide to glycolide used for the polymerization, different forms of PLGA can be obtained: these are usually identified in regard to the monomers' ratio used (e.g. PLGA 75:25 identifies a copolymer whose composition is 75% lactic acid and 25% glycolic acid. All PLGAs are *amorphous* rather than *crystalline* and show a *glass transition temperature* in the range of 40-60 °C.

PLGA can be dissolved by a wide range of common *solvents*, including *chlorinated* solvents, *tetrahydrofuran*, *acetone* or *ethyl acetate*.

PLGA degrades by *hydrolysis* of its ester linkages in the presence of *water*. It has been shown that the time required for degradation of PLGA is related to the monomers' ratio used in production. The higher the content of glycolide units, the lower the time required for degradation. An exception to this rule is the copolymer with 50:50 monomers' ratio which exhibits the faster degradation (about two months).

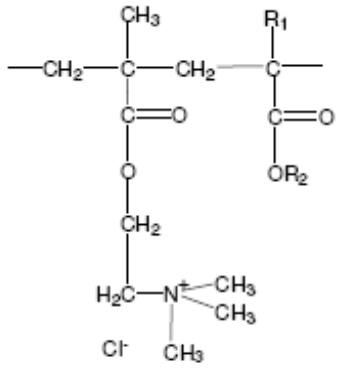
PLGA has been successful as a biodegradable polymer because it undergoes hydrolysis in the body to produce the original monomers, lactic acid and glycolic acid. These two monomers under normal physiological conditions are by-products of various *metabolic pathways* in the body. Since the body effectively deals with the two monomers, there is very minimal systemic *toxicity* associated with using PLGA for drug delivery or biomaterial applications. (www.wikipedia.com).

2. EUDRAGIT RL 100

Commercial form

Eudragit RL 100/ RS 100

Structure



R_1 = H, CH_3
R_2 = CH_3, C_2H_5

CHARACTERS

Description

Colour

Colurless, clear to cloudy granules with a faint amine-like colour

Solubility

Soluble in ethanol, isopropyl alcohol,acetone and methylene chloride. Practically insoluble in petroleum ether,water.

Particle size

At least 90% < 0.315 mm.

Film formation

When the test solution is poured onto a glass plate a clear film forms upon evaporation of the solvents

Dry substance / Residue on evaporation

Not less than 97.0%.1 gm of the substance is dried in an oven for 5 hours in vaccum at 80° C.

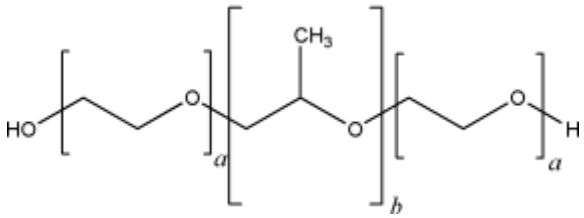
Loss on drying	:	Maximum 3%
Viscosity/ Apparent viscosity	:	Maximum 15mPa.s
Refractive index	:	n_D^{20}.1.380-1.385
Relative density	:	0.816-0.836

Storage

Protect from warm temperatures,Protect from moisture. (www.wikipedia.com)

3. POLOXAMER 188

Structure



Nonproprietary names

BP : Poloxamers

PhEur : Poloxamera

USPNF : Poloxamer

Synonyms

Lutrol, Monolan, Pluronic, Symperonic, Supronic and Poloxalkol

Chemical name and CAS registry number

α-Hydro-co-hydroxypoly (oxyethylene) poly (oxypropylene)poly (oxy ethylene) block copolymer

$HO(C_2H_4O)a(C_3H_6O)b(C_2H_4O)aH$

CAS No. : 9003-11-6

Molecular weight : 7680-9510

Functional category

Dispersing agent; emulsifying agent; solubilizing agent; tablet lubricant; wetting agent.

Description

Poloxamers generally occur as white, waxy, free flowing prilled granules, or as cast solids. They are practically odorless and tasteless.

Density	:	1.06 g/cm^3 at 25° C
HLB Value	:	29
Melting point	:	52-57° C
Solubility	:	Freely soluble in ethanol and water
Surface tension	:	19.8 mN/m (19.8 dynes/cm) for a 0.1%w/v aqueous Poloxamer solution at 25° C
Viscosity	:	1000 mPas (1000 cP), melts at 77° C

Stability and storage conditions

Poloxamers are stable materials. Aqueous solutions are stable in the presence of acids, alkalis, and metal ions. However aqueous solutions support mould growth.

The bulk materials should be stored in a well-closed container in a cool, dry place.

Incompatibilities

Poloxamer 188 is incompatible with phenols and parabens.

Method of manufacture

Poloxamer polymers are prepared by reacting propylene oxide with propylene glycol to form polyoxypropylene glycol. Ethylene oxide is then added to form the block polymer.

Safety

Poloxamers are used in a variety of oral, parenteral and topical pharmaceutical formulations and are generally regarded as nontoxic and non-irritant materials. Poloxamers are not metabolized in the body. (Raymond.C.Rower et al., 2003).

Applications of Poloxamer in pharmaceutical field

Use	Concentration (%)
Wetting agent	0.01-5
Tablet excipients	5-10
Tablet coating	10
Stabilizing agent	1-5
Gelling agent	15-50

MATERIALS AND METHODS

Materials used for the preparation and evaluation of 5-Fluorouracil nanoparticles

Materials	Grade	Source
5-Fluorouracil	Extra pure, RM-1502	Himedia Laboratories Pvt. Ltd, Mumbai
Eudragit	RL 100	Ozone International, Mumbai
Hydrochloric acid	-	Lobachemie Ltd, Mumbai
Methanol	-	Lobachemie Ltd, Mumbai
Potassium dihydrogen phosphate	-	Himedia Laboratories Pvt. Ltd, Mumbai
Disodium hydrogen phosphate	-	Lobachemie Ltd, Mumbai
Sodium hydroxide	LR	Spectra reagent & Chemicals Pvt. Ltd
Distilled water	Double distilled	Aqua Purification Systems, Salem
Diethyl Phthalate	-	Lobachemie Ltd, Mumbai
Acetone	-	Lobachemie Ltd, Mumbai
Dichloromethane	-	Lobachemie Ltd, Mumbai
PLGA	50:50, 25:75	Sigma Aldrich, Germany
Dibutyl phthalate	-	Lobachemie Ltd, Mumbai

EQUIPMENTS USED

Equipments used for the preparation of nanoparticles

Equipment	Model/company
Freeze Drier	Labconco,USA
Scanning Electron Microscope	Jeol Jsm-6400
Dissolution test apparatus	Digital dissolution test Apparatus, Lab India.
UV Visible spectrophotometer	UV-1650 PC
FT/ IR Spectrometer	FT IR--8400S(CE),SHIMADZU
IR-hydraulic Pellet Press	M-15, KBr press,
Digital Balance	H.R- 200, A&D – company Ltd.
Rotary Flask Evaporator	Double Coiled Condenser, Superfit
pH Meter	LI- 120, ELICO
Magnetic Stirrer	Remi Equipments Ltd
Vaccum dessicator	-
Vortex Mixer	CM 101 Cyclo Mixer
Mechanical Stirrer	RQ-122, Remi Equipments Ltd
Water Purifier	Model No.2874/06, Tka Ltd

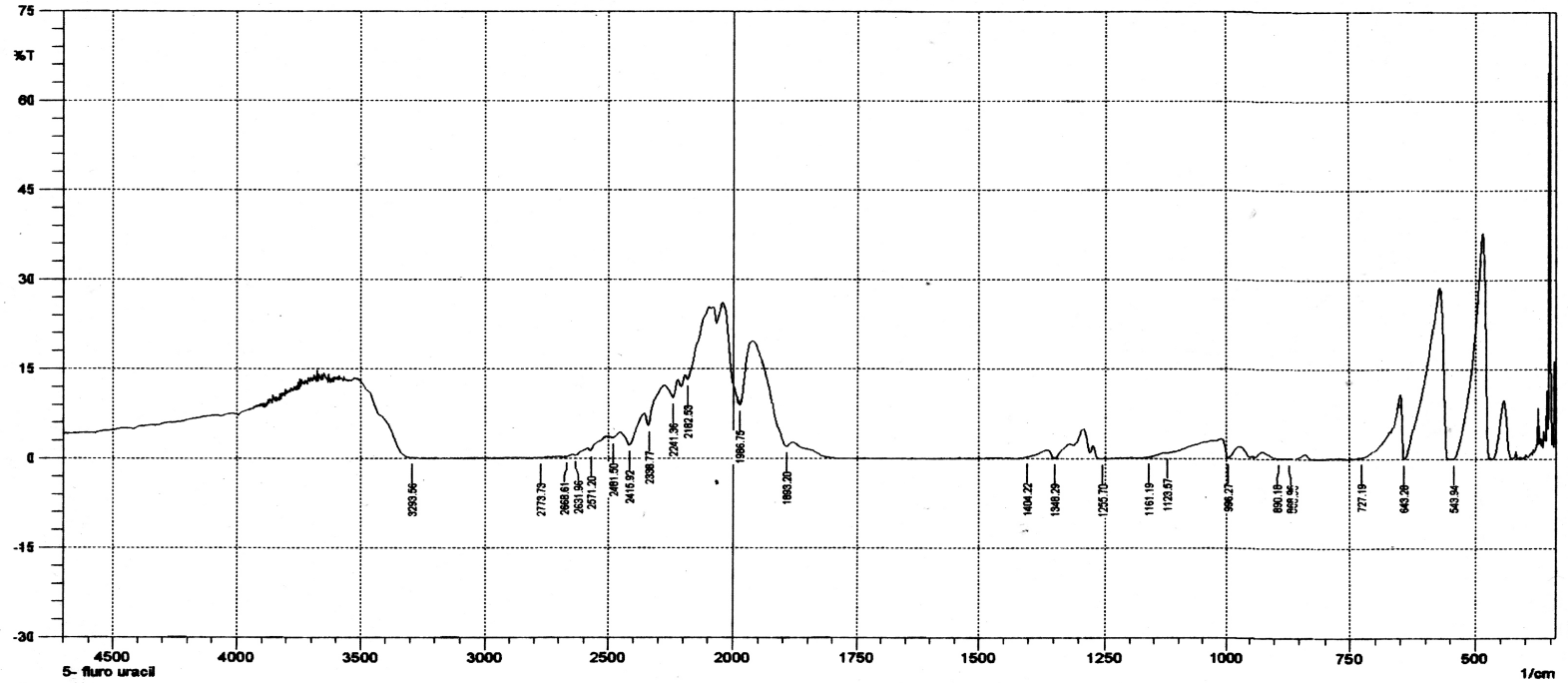
ANALYTICAL METHODS

Preformulation studies

Infrared (IR) absorption spectroscopy

To investigate any possible interactions between the drug and the polymers, the IR spectra of pure 5-Fluorouracil, PLGA and its physical mixtures (1:1) with PLGA were carried out using Shimadzu FT IR 8400 S (CE) spectrophotometer (Tokyo, Japan). The samples were prepared as KBr pellets compressed under a pressure of 6 ton /nm^2. The wavelength selected ranged between 400 - 4000cm^{-1} in a Perkin Elmer FTIR spectrophotometer. The IR spectrum of the physical mixture was compared with those of pure drug and polymer, matching was done to detect any appearance or disappearance of peaks.

Fig. 3 IR SPECTRUM OF 5 -FLUOROURACIL



Comment;
5-fluro uracil

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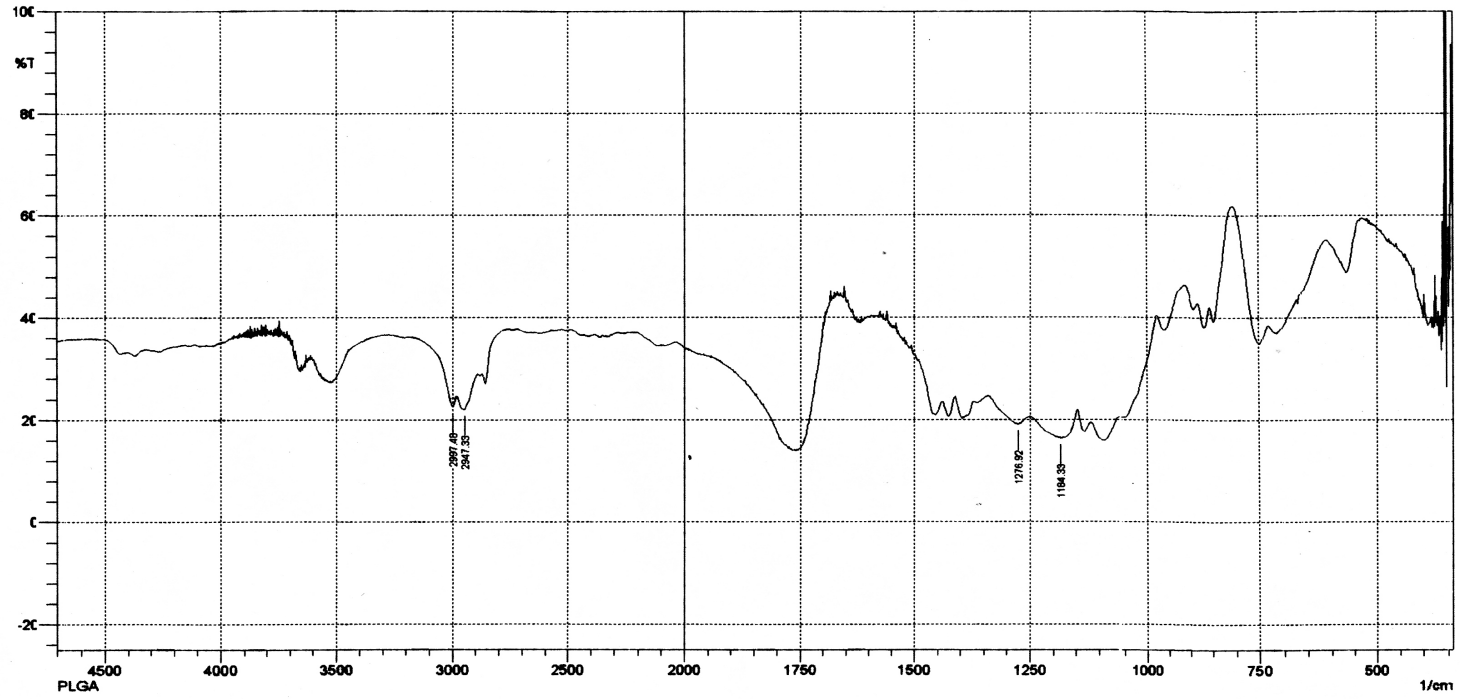
Resolution; 2 [1/cm]
Apodization; Happ-Genzel

Date/Time; 01/29/2009 03:24:00 PM
User; Administrator

Fig. 4 IR SPECTRUM OF PLGA

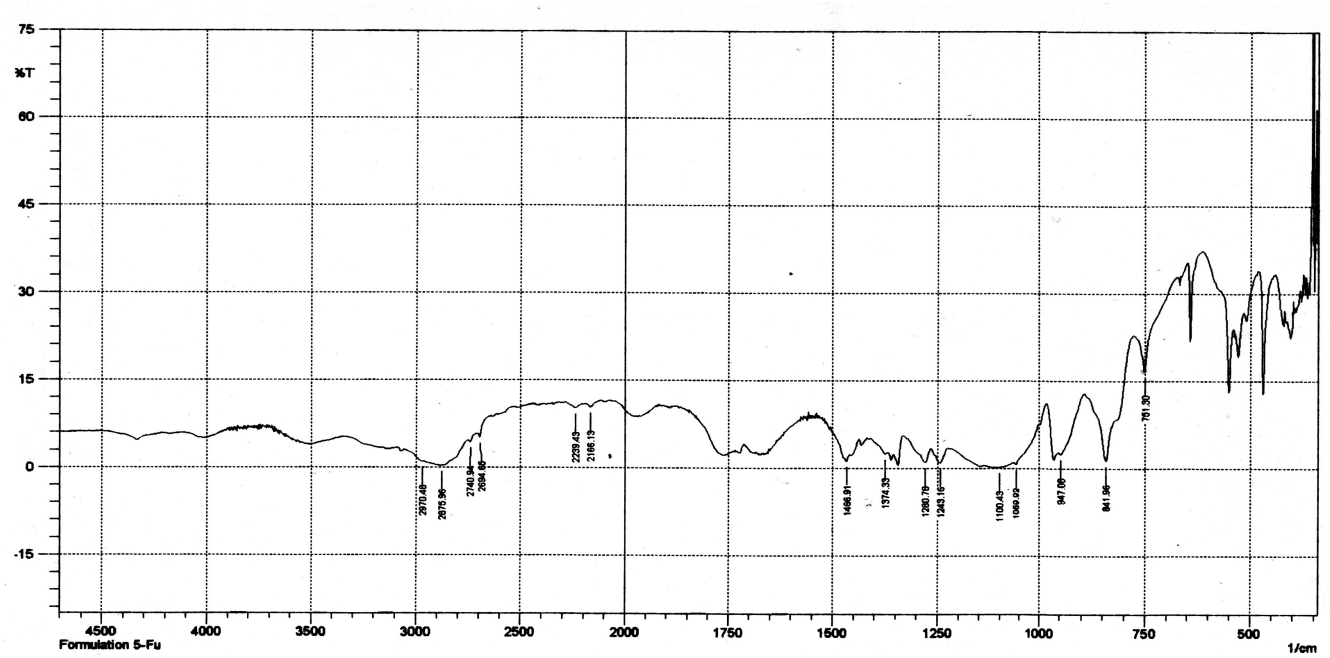


Comment;
PLGA

No. of Scans; 10
Resolution; 2 [1/cm]
Apodization; Happ-Genzel

Date/Time; 01./29/2009 03:59:08 PM
User; Ad ministrator

Fig. 5 IR SPECTRUM OF OF PHYSICAL MIXTURE OF 5 -FU + PLGA (1:1)



PREPARATION OF STANDARD GRAPH OF 5-FLUOROURACIL USING PHOSPHATE BUFFER pH 7.4

Accurately weighed quantity of 100 mg of 5-Fluorouracil was transferred in to a 100 ml standard flask and volume is made up to the level of standard flask with phosphate buffer (pH.7.4). From the primary stock solution 2 ml was diluted to 100 ml with phosphate buffer (pH 7.4) in order to produce a concentration of 20 µgm/ml solution. The above working solution was subsequently diluted with respective buffer to obtain a series of dilutions of 2µgm/ml, 4µgm/ml, 6µgm/ml, 8µgm/ml, 10µgm/ml, 12µgm/ml, 14µgm/ml, 16µgm/ml, 18µgm/ml and 20µgm/ml. The absorbance of the above solutions was measured in UV-visible spectrophotometer at 266 nm using their blank.

PREPARATION OF STANDARD GRAPH OF 5-FLUOROURACIL USING PHOSPHATE BUFFER pH 6.8

Accurately weighed quantity of 100 mg of 5-Fluorouracil was transferred in to a 100 ml standard flask and volume is made up to the level of standard flask with phosphate buffer (pH.6.8). From the primary stock solution 2 ml was diluted to 100 ml with phosphate buffer (pH 6.8) in order to produce a concentration of 20 µgm/ml solution. The above working solution was subsequently diluted with respective buffer to obtain a series of dilutions of 2µgm/ml, 4µgm/ml, 6µgm/ml, 8µgm/ml, 10µgm/ml, 12µgm/ml, 14µgm/ml, 16µgm/ml, 18µgm/ml and 20µgm/ml. The

absorbance of the above solutions were measured in UV-visible spectrophotometer at 266nm using the blank.

PREPARATION OF STANDARD GRAPH OF 5-FLUOROURACIL USING PHOSPHATE BUFFER pH 1.2

Accurately weighed quantity of 100 mg of 5-Fluorouracil was transferred in to a 100 ml standard flask and volume is made up to the mark with hydrochloric acid buffer (pH-1.2). From the primary stock solution 10 ml was diluted to 100 ml with hydrochloric acid (pH-1.2) in order to produce a concentration of 100 µgm/ml solution. The above working solution were subsequently diluted with respective buffer to obtain a series of dilutions of 10µgm/ml, 20µgm/ml, 30µgm/ml, 40µgm/ml and 50µgm/ml. The absorbance of the above solutions were measured in UV-visible spectrophotometer at 266nm using buffer as blank.

Table 1: Standard graph of 5-Fluorouracil at pH 7.4

S. No	Concentration (mcg/ml)	Absorbance at 266 nm
1	2	0.11536
2	4	0.20630
3	6	0.29843
4	8	0.39001
5	10	0.51038
6	12	0.59875
7	14	0.70923
8	16	0.77332
9	18	0.91687
10	20	0.99768

Fig. 6 : Standard graph of 5-Fluorouracil at pH 7.4

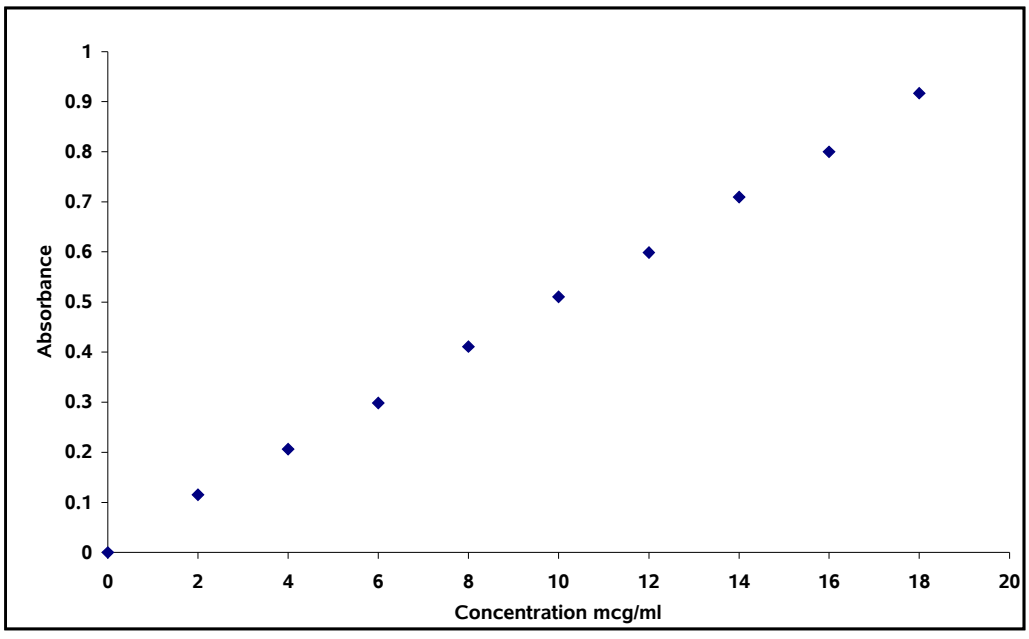


Table 2 : Standard graph of 5-Fluorouracil at pH 6.8

S. No	Concentration (mcg/ml)	Absorbance at 266 nm
1	2	0.12695
2	4	0.2616
3	6	0.37317
4	8	0.50537
5	10	0.61682

Fig. 7 : Standard graph of 5-Fluorouracil at pH 6.8

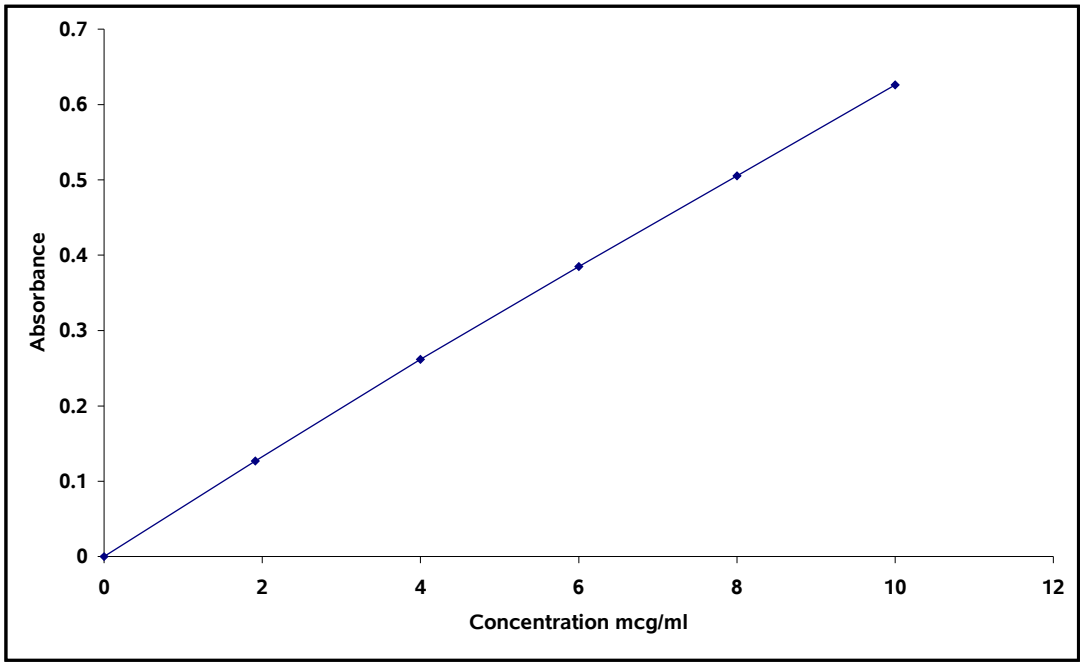
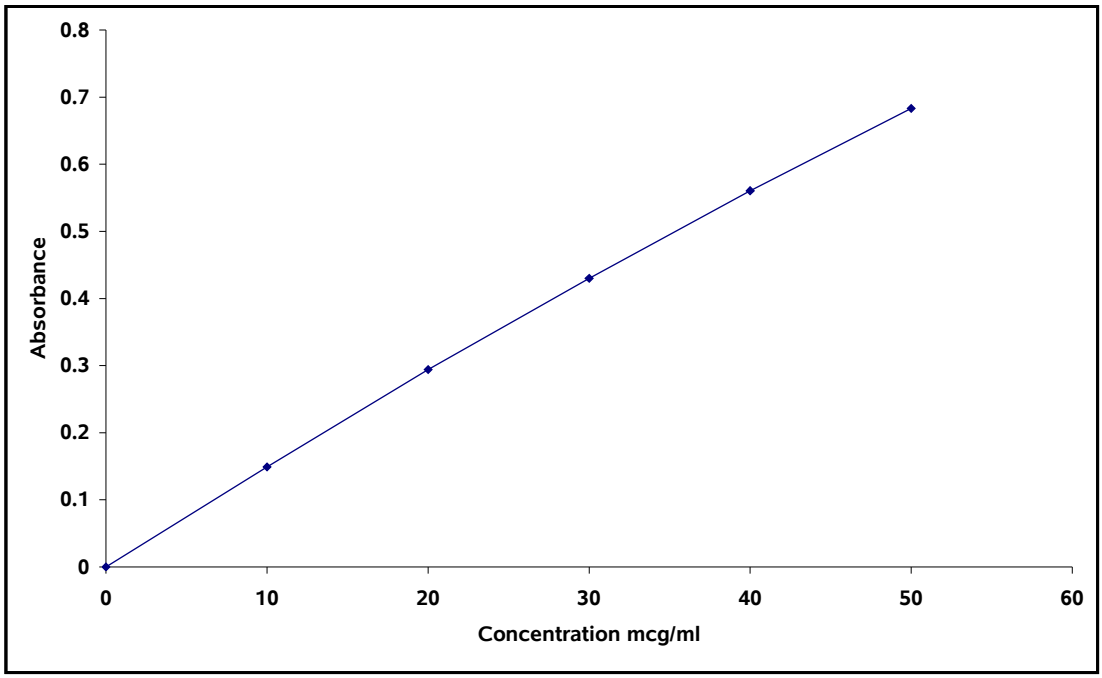


Table 3 : Standard graph of 5-Fluorouracil at pH 1.2

S. NO	Concentration (mcg/ml)	Absorbance at 266 nm
1	10	0.15222
2	20	0.25825
3	30	0.43005
4	40	0.56055
5	50	0.68311

Fig. 8 : Standard graph of 5-Fluorouracil at pH 1.2



METHODOLOGY

Preparation of drug loaded nanoparticles

Drug loaded NP were prepared using a nanoprecipitation method. An accurately weighed quantity of 100mg PLGA was dissolved in 10 ml of acetone and accurately about 4.0 mg of 5-Fluorouracil was weighed and dissolved in the polymer solution was poured immediately into a stirred, aqueous solution of 0.5% (w/v) Poloxamer 188 previously filtered through 0.22 mm poly (carbonate) filter. The resulting cloudy suspension was transferred expeditiously to a round –bottomed flask and evaporated under reduced pressure for 20 minutes at 40°C. The product was then freeze dried. (McCarron et al., 2008).

Table 4 : Preparation of 5-Fluorouracil Nanoparticles

S. No	Formulation code	Drug	Polymer
1	NP-1	5-Fu	PLGA (50: 50)
2	NP-2	5-Fu	PLGA (25:75)

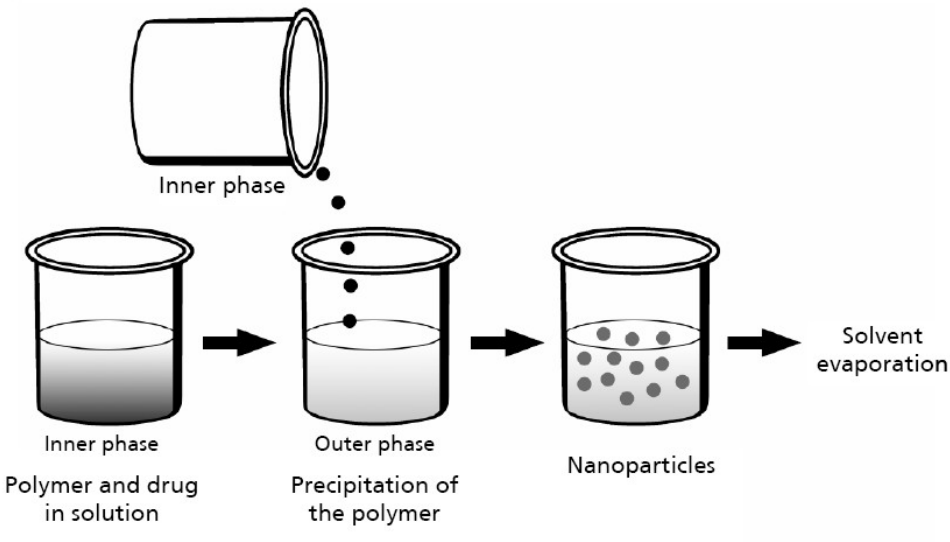


Fig. 9 : Preparation of Nanoparticles

SCANNING ELECTRON MICROSCOPE (SEM) OBSERVATION

The morphology of obtained particles of 5-Fluorouracil nanoparticles was examined by scanning electron microscope (SEM) JEOL JSM-6400 at 30 KV magnification at room temperature. Before scanning nanoparticle sample were sputtered with platinum (HITACHI, E-1010, ION SPUTTER) (Fig. 10 and 10A), shows the magnification of sample at 2000 and 3000 .

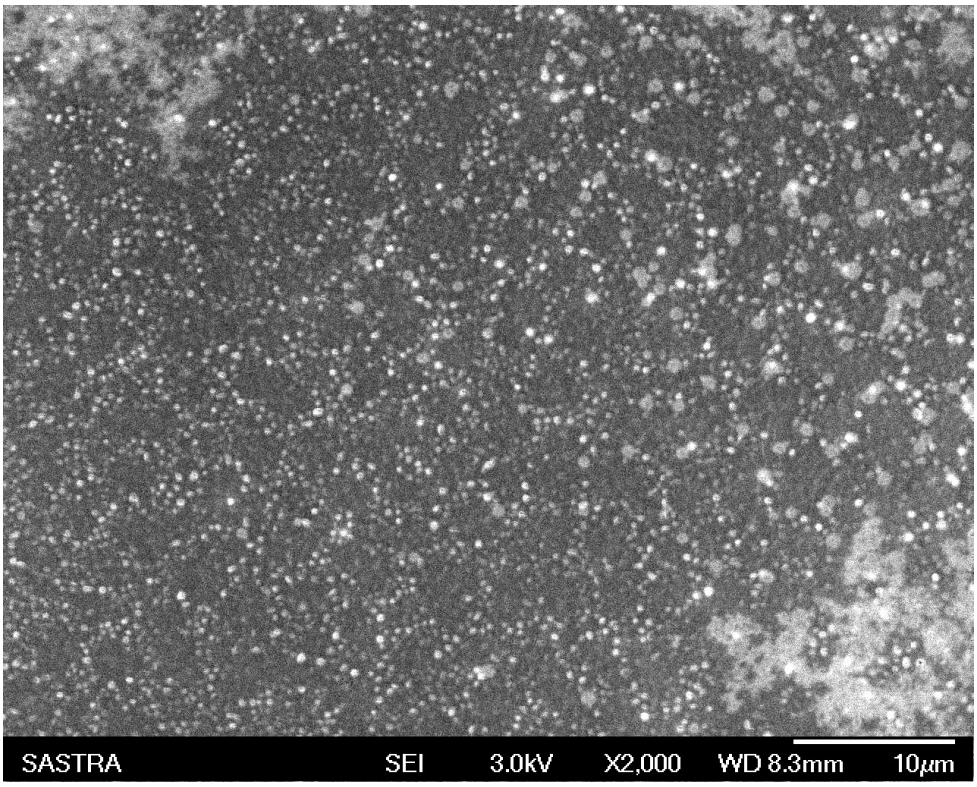


Fig. 10: X- 2000 Magnification

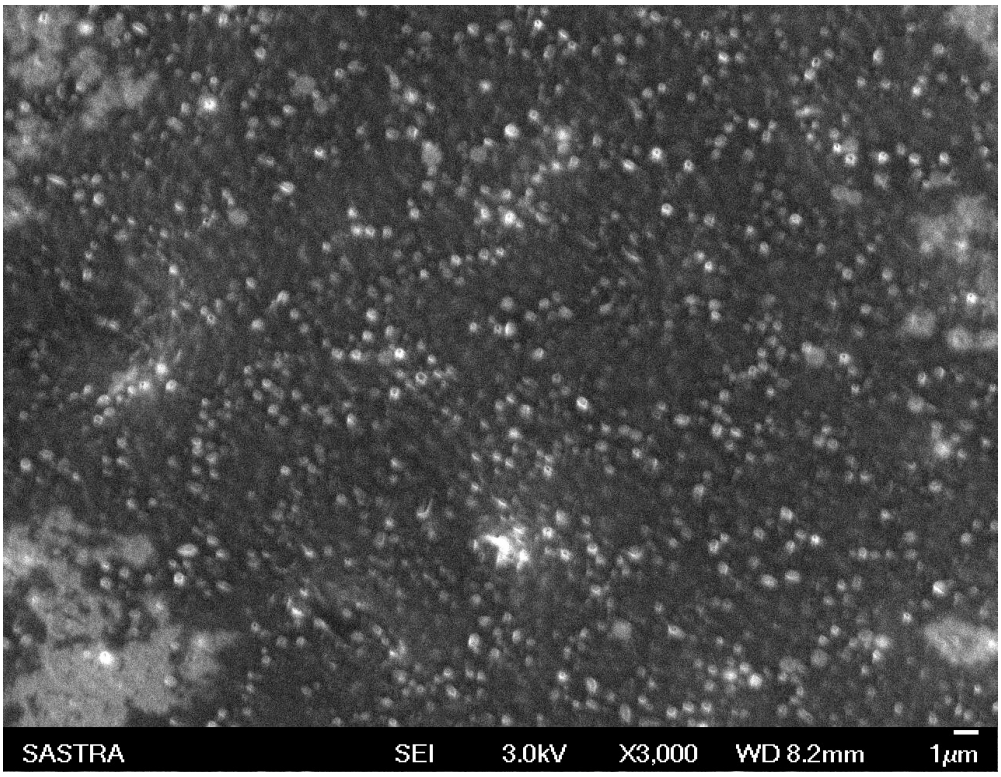


Fig. 10A : X -3000 Magnification SEM Images

DETERMINATION OF 5-FU CONTENTS OF NANOPARTICLES

Five milliliters of methylene chloride was added to 30 mg of lyophilized 5-FU-nanoparticles (5Fu-NP) for digestion and 10 ml of bi-distilled water was added in order to extract the 5-Fu. The aqueous layer was filtered through a membrane filter and assayed spectrophotometrically at 266 nm. The entrapping efficiency (EE) of 5Fu- NP was calculated using the equation (Asuman and Ongum, 2005).

EE (%) = [(a × b)/c] × 100

NP - 1

a - Weight of nanoparticle;

b-Drug content;

c- Added drug amount.

Weight of the nanoparticle = 30mg

Drug content in the nanoparticle = 0.02185mg

Added drug amount = 0.85714mg

Entrapping Efficiency EE (%) = $\frac{30 \text{ X } 0.02185 \text{ X } 100}{0.85714}$

= 76.47 %.

==========

NP- 2

Weight of the nanoparticle = 30mg

Drug content in the nanoparticle = 0.00125mg

Added drug amount = 0.85714mg

Entrapping Efficiency EE (%) = $\frac{30 \text{ X } 0.001255 \text{ X } 100}{0.85714}$

= 4.37 %

==========

TARGETING OF THE NANOPARTICLES TO COLON

Preparation of Eudragit RL 100 coated gelatin capsule

Dipping method

The different concentrations of Eudragit RL100 solutions were prepared (4%. 6%, 8% and 10%) by mixing the powder Eudragit RL 100 with methanol and dichloromethane mixture in a ratio of 1:4. Dibutyl phthalate (2 % v/v) was also added to the above solution as plasticizer. The hard gelatin empty capsules (size No.1) were taken and separated the cap and the body, each part is dipped into different ratio solutions Eudragit RL100. Six times the procedure was repeated in a time interval of 3 hours. The 5-Fu nanoparticles are filled in the capsules and sealed.

In Vitro Drug Release studies of the Eudragit RL 100 coated capsules

Nanoparticles equivalent to 100mg of 5-Fu were filled in a capsule and sealed with Eudragit RL100 and dissolution studies carried out in 900 ml of pH medium at 37^0C in 60 rpm. The pH of the medium was gradually increased from 0.1 N HCl (pH1.2), Phosphate buffer pH 5.8, pH 6.8 respectively for 2 hours each, then transferred it to pH 7.4 phosphate buffer until the end of the experiment. At specific time interval 1,2….11 hours, dissolution sample was quantified spectrophotometrically at 266nm.

Table 5: Eudragit RL 100 (4% w/v) In vitro drug release

S.No	Time in minutes	Percentage release
1	300	00.00
2	315	08.07 ± 0.62
3	330	28.65 ±0.83
4	345	39.85 ±0.28
5	360	44.55 ±0.59
6	375	52.21 ±0.60
7	390	58.65 ±0.70
8	405	64.86 ±0.71
9	420	77.02 ±0.72
10	435	85.75 ±0.45
11	450	96.10 ±0.46

Fig. 11 Eudragit RL 100 (4% w/v) In vitro drug release

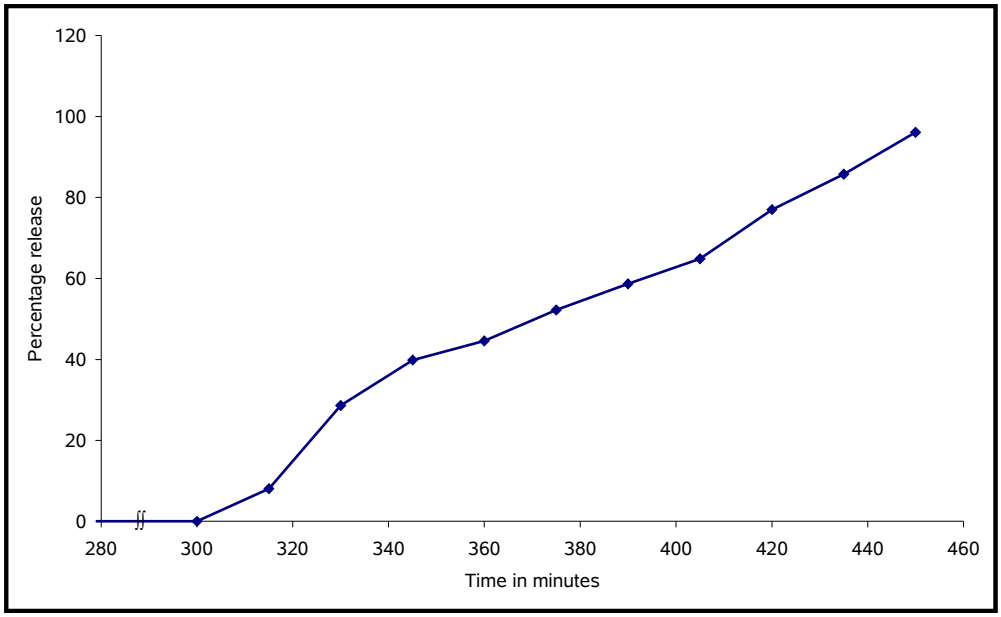


Table 6: Eudragit RL 100 (6% w/v) In vitro drug release

S.No	Time in hours	Percentage release
1	390	00.00
2	405	02.06 ±0.28
3	420	12.57 ±0.46
4	435	26.05 ±0.58
5	450	49.00 ±0.8
6	465	53.46 ±0.38
7	480	76.65 ±0.32
8	495	81.75 ±0.18
9	510	88.06 ±0.33
10	525	92.45 ±0.37
11	540	93.33 ±0.27

Fig. 12 Eudragit RL 100 (6% w/v) In vitro drug release

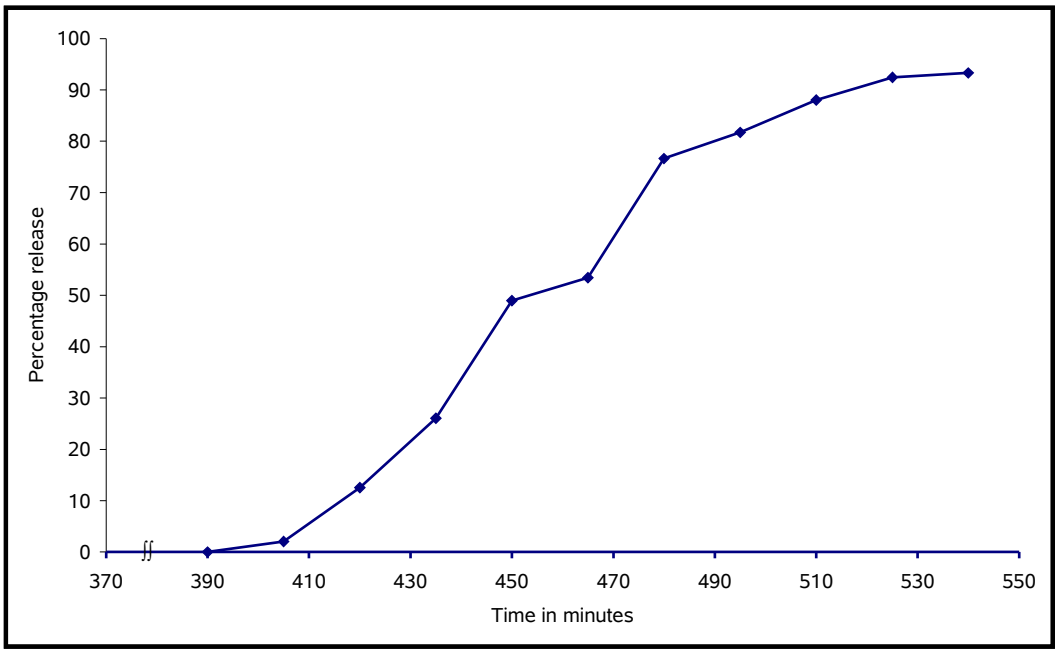


Table 7: Eudragit RL 100 (8% w/v) In vitro drug release

Fig. 13 Eudragit RL 100 (8% w/v) In vitro drug release

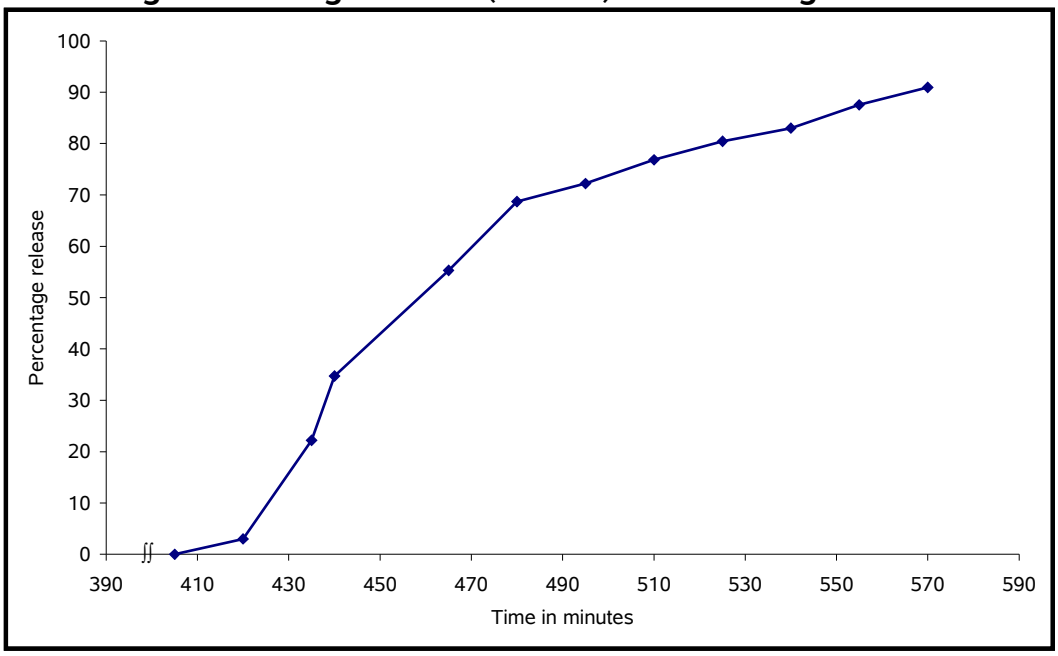
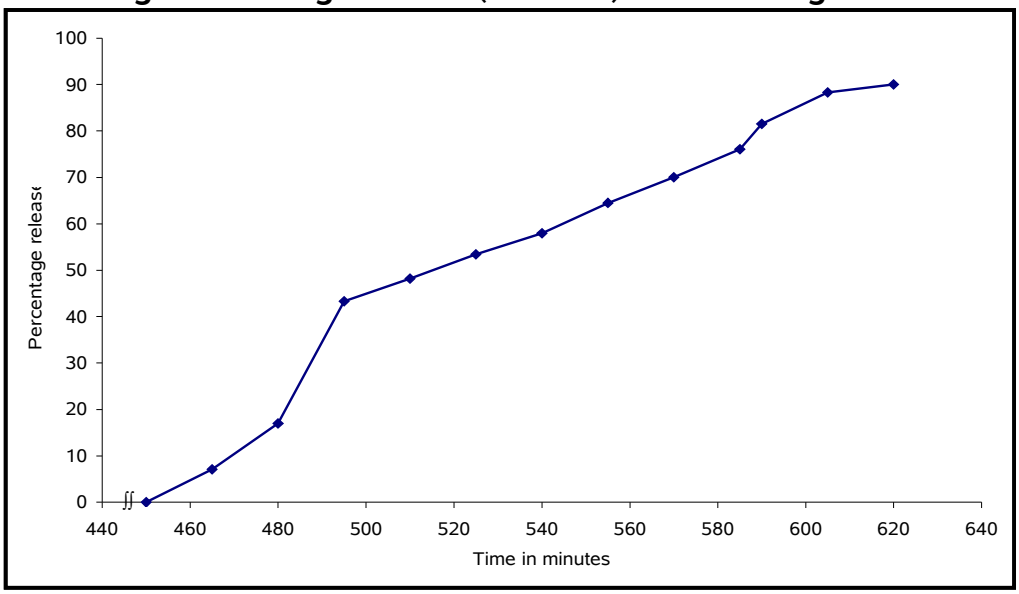


Table 8 : Eudragit RL 100 (10% w/v) In vitro drug release

S. No	Time in hours	Percentage release
1	450	00.00
2	465	07.09 ± 0.23
3	480	16.99± 0.22
4	495	43.33± 0.46
5	510	48.21± 0.19
6	525	53.43± 0.27
7	540	57.98± 0.90
8	555	64.47± 0.92
9	570	70.07± 0.95
10	585	76.05± 0.92
11	590	81.54± 0.88
12	605	88.31± 0.84

13	620	89.99± 0.69

Fig. 14 Eudragit RL 100 (10% w/v) In vitro drug release



RESULTS AND DISCUSSION

FT-IR SPECTRAL ANALYSIS

Compatibility studies of 5-Fluorouracil, PLGA (50:50) were carried out by FT-IR peak matching method. The IR spectra of pure drug, polymer and physical mixture (1:1) shown in Fig 3, 4 and 5 respectively.

The IR spectrum of physical mixture did not show any significant differences from those obtained for pure samples. These obtained results indicate that there was no positive evidence for the interaction between the drug and the polymer. More than hydrogen bonding (if any), which may have occurred between donating and accepting groups of both drug and polymer.

PARTICLE SIZE AND SHAPE ANALYSIS USING SEM

Scanning Electron Microscopy reveals that all prepared nanoparticles had a homogeneous solid matrix structure, with no evidences of crystals on the surface (Fig.10 and 10A).The PLGA loaded nanoparticles with 5-Flurouracil produced by nanoprecipitation method enabled us to get spherical, discrete spheres with a size ranging from 300 – 500nm.

ENCAPSULATION EFFICIENCY STUDIES

Encapsulation efficiency (EE) of nanoparticles was found to be 76.47% and 4.38% for the formulation NP-1 and NP-2 respectively. The encapsulation efficiency of a nanoparticulate carrier is mainly altered by the nature drug incorporated, and the method of production adopted. The results indicated the formulation NP-1 has a good encapsulation efficiency, which revealed that this drug and polymer ratio is ideal for formulations.

IN VITRO DRUG RELEASE STUDIES OF COLON TARGETED EUDRAGIT RL 100 COATED NANOPARTICLES

The characterization of in vitro drug release from a colloidal carrier especially under sink condition is technically difficult to achieve. This could be attributed to the inability to separate successfully the particles from the dissolved or release drug in the sink solution due to the very small size of the particles. Attempting to evaluate the drug release from colloidal carriers, diverse techniques have been used for example, diffusion cells, where the undiluted colloidal drug carrier is separated from the sink solution by porous membrane.

As an acidic polymer Eudragit RL 100 is ionized to a greater extent at higher pH, accounting for a greater degree of hydration. Dissolution study of Eudragit RL 100 coated capsules in simulated pH 1.2 and pH 5.8, revealed that there is no release of 5-Fu from all four different percentage of Eudragit coatings. Eudragit RL 100 coated capsules are showing a good release of drug in the pH of 6.8 and 7.4. Release rate of 5-Fu form Eudragit RL 100 was found to be pH dependent. In four concentrations the 10% solution used for coating the capsules were showing good release pattern of 5-FU in a delayed rate (Fig.14). So, the 10 % concentration of Eudragit RL 100 coated capsules is suitable for colon targeting.

SUMMARY AND CONCLUSION

The nanoprecipitation procedure was used to prepare biodegradable nanoparticles of spherical shape and size range of 300-500 nm with different of processing parameters, the results of the in vitro release profiles of the entrapped anticancer drug from the capsule were pH dependent erosion of the Eudragit RL 100 layer according to the different area of the intestine. The designed site specific delivery of 5-Fluorouracil capsules may reduce the side effects of the drug caused by its absorption from the GI tract when given in conventional dosage forms.

The results of this study clearly indicate that there was great potential in delivery of 5-Fluorouracil to the colonic region. So it is an alternative to the conventional dosage form. However, more extensive pharmacokinetic and pharmacodynamic studies are needed before establishing colonic delivery of Eudragit coated 5-Fluorouracil capsules as an alternative. Eudragit RL 100 is a biocompatible polymer; we expect it to cause no harmful effects if used for prolonged periods. This study has suggestes that the Eudragit RL 100 coated capsules offer an excellent potential for the targeted release of drugs for colorectal cancer.

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